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# PHYTOPATHOLOGY

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NUMBER 1

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*Methods of Spore Measurement in the Actinomyces.* F. M. BLODGETT.

From spore measurements, alone, strains of *Actinomyces*, isolated from potato-scab lesions and found to be pathogenic, can be separated into numerous groups. To make the measurements an apparatus was designed that consists of a filar micrometer operated by a flexible shaft, which makes it possible to avoid disturbing the focus of the microscope during a measurement, as so easily occurs with the ordinary filar micrometer. The apparatus as built proved adequate and convenient for its purpose. A standard synthetic culture medium proved essential, as did also a rather elaborate method of sampling, if successive samples were to agree as closely as expected. The measurements did not fit a normal frequency curve, but the curves were more or less skewed. However, it was found that with an adequate sampling technique, even though the curves were skewed, the usual constants, the standard deviation, and the mean and standard error of the means were fairly reliable guides. The *chi-square* test did provide a somewhat more satisfactory method, inasmuch as it compares the frequencies in all parts of the range of sizes, and it is based on no assumption as to the shape of the frequency distribution involved.

*Stimulation of Potatoes by Magnesium Bordeaux Spray.* REINER BONDE.

Spraying potatoes with Bordeaux mixture prepared with a high-calcium hydrated lime has failed to stimulate plant growth and to increase yield in Aroostook County, Maine, when applied in the absence of insects and diseases. Bordeaux, prepared with a hydrated lime high (about 30%) in magnesium, has stimulated potato-plant growth and yield on Aroostook soil deficient in magnesium. Magnesium deficiency causes general stunting, chlorosis, and necrosis of the lower leaves, along the veins, which may involve the whole plant. Deficient plants sprayed with magnesium Bordeaux showed recovery in the new growth and lived longer. In one test the yield increase was 96 bushels an acre, or 343 per cent of the yield of 28 bushels secured in adjacent plots sprayed with calcium Bordeaux. On less deficient soil in the same field, the yield was increased only 15 per cent of the 253 bushels per acre secured with calcium Bordeaux. In another test the magnesium Bordeaux increased the yield 133 per cent on deficient soil and 11 per cent on better soil. It is suggested that some spray stimulation reported in the past may have been due to magnesium absorption.

*Strawberry Dwarf in Massachusetts.* O. C. BOYD, J. R. CHRISTIE and N. E. STEVENS.

Strawberry dwarf (*Aphelenchoides fragariae*) was first observed in Massachusetts in 1931. There were then found only traces of infection in a few beds of the Blakemore and Howard 17 (Premier) varieties. In 1932 and 1933, dwarf was more widespread, the symptoms more pronounced and the injury much greater. Losses in individual fields of the Howard 17 variety ranged up to 50 per cent of plants killed. Seasonal behavior of diseased plants is different in Massachusetts and in the south. In most of the southern States symptoms of dwarf are conspicuous in summer and the plants are nearly or quite normal during the spring. On Cape Cod dwarf symptoms are most conspicuous in the spring. Both in seasonal relations and appearance of severely diseased plants the disease, in Massachusetts, resembles "Cauliflower" of strawberries in Europe (caused by the same nematode) rather than typical "dwarf" in this country. The nematode population of diseased plants in Massachusetts is very high. One plant, examined early in May, was found to harbor 11,000 *A. fragariae*. In contrast the highest nematode population on dwarfed plants in North Carolina, examined weekly over a period of nearly 2 years, was about a thousand.

*A Mosaic Disease of Bulbous Iris.* PHILIP BRIERLEY and FRANK P. MCWHORTER.

The establishment of commercial Spanish and Dutch bulbous-iris plantings in America has been hindered by a destructive mosaic disease, introduced on the stocks imported from Europe. Infected plants are dwarfed, develop yellow-striped or mottled leaves and flower sheaves and blotched flowers. Investigations, 1929-1933, prove it a transmissible mosaic disease, with results as follows: Varietal and intervarietal tissue grafts (diseased tissue inserted into slits in healthy stems), 42 trials totaling 453 plants averaged 41 per cent mosaic transfer; hypodermic-needle injections into nodes, 37 trials, 477 plants, averaged 12 per cent; into internodal pith, 5 trials, 46 plants averaged 56 per cent. Inoculation by single and multiple needle and by rubbing methods has not been successful. Flower and pod cutting tests were negative, indicating little danger of accidental mechanical transfer during ordinary cultural operations. Natural transmission appears to be due to aphids. Cage tests gave the following results: *Myzus persicae*, 4 trials, 50 plants averaged 50 per cent transmission; *Illinoia solanifolii*, 14 trials, 122 plants averaged 31 per cent. Tests were negative with *Myzus pelargonii* and *M. circumflexus* and with the "bulb aphids" *Anuraphis tulipae* and *Rhopalosiphoninus tulipaella*. Transfer by aphids occurred only where the insects migrated naturally from diseased to healthy plants within the cage. All infection experiments were checked by noninoculated sister-bulb controls and nonviruliferous aphids.

*Attempted Removal of Staling Substances of Fungus Cultures.* J. C. CARTER.

*Helminthosporium sativum* and a bacterial organism designated as 9a<sub>2</sub> are used to stale the medium. Fifty c.c. of nutrient broth are placed in 250 c.c. flasks and inoculated with *Helminthosporium sativum*. Two days later the same nutrient broth is inoculated with 9a<sub>2</sub>. The cultures are incubated for 10 days in the dark at room temperature. The staled medium is filtered through filter paper and Berkefeld filters. The filtrate is divided into 2 equal portions. Portion No. 1 is dialized in a Bradfield dializer for 45 minutes. Portion No. 2 is not dialized but is otherwise treated as is No. 1. Nutrient broth is added to the water accumulated in the + and - compartments of the dializer; pH readings are taken on all materials obtained before and after dialysis. A half of the solution obtained from each of the 3 compartments is reinoculated with *H. sativum*. The other half of the various solutions is reinoculated only after the pH value of the original broth (pH 6.86) has been restored. All solutions are sterilized before reinoculation. Sterilization does not inactivate the staling agent. Filtered broth inoculated with *H. sativum* serves as a check. Readings showing the percentage of fungus growth are taken after 6 days. Results indicate that the staling agent is attracted to the negative pole of the Bradfield dializer.

*Fungi and Bacteria on Barley.* J. J. CHRISTENSEN and E. C. STAKMAN.

Barley plants were inoculated with 200 isolates from 8 genera of fungi; many of them caused leaf and sheath lesions as well as discolored kernels. Definite symptoms cannot be assigned to individual organisms, as there is great variation in symptoms, depending on environmental factors, varieties affected, the stage of development of the host, and the association of various organisms. Secondary invasions, especially by *Alternaria* spp., are common. Several bacterial isolates also were pathogenic. Isolations were made from about 500 samples of barley seed, mostly from Minnesota, and fungi belonging to more than 20 genera were isolated. In 1932 *Fusarium* spp. and *Helminthosporium* spp. were responsible for about 50 per cent of the shriveling and discoloration of kernels and, in 1933, for approximately 30 per cent. Evidently many other organisms

are important in causing defects on barley seeds. In both years *Helminthosporium* was more prevalent than *Fusarium*. Treating diseased seed lots with Ceresan resulted in improved stands and often in increased yields. Germination of seed on blotters is not a safe indication of performance in soil. (Minnesota Agricultural Experiment Station.)

*Progress in the Control of Tobacco Downy Mildew.* E. E. CLAYTON and J. G. GAINES.

Attempts to escape infection by locating beds on virgin land in remote places, sterilizing the soil, or treating the seed, have been ineffective. Tests of a large number of varieties of *Nicotiana tabacum* have shown all to be susceptible, with a Turkish type (*Xanthia*) least affected. *N. rustica* proved resistant. Recovered plants were distinctly more resistant to the disease than were plants not previously attacked. Extensive spraying experiments have included consideration of schedules, methods of application, and materials. Among materials tested were the following sprays: Bordeaux, copper-soap, ammoniacal copper carbonate, copper acetate, oxide, sulphate, silicate, phosphate, and resinate, colloidal sulphur, potassium sulphide, calcium monosulphide, phenyl mercury acetate, oxyquinoline sulphate and cottonseed oil. Dusts used were: Bordeaux, copper-lime, copper carbonate, copper stearate, sulphur, calomel, and ethyl mercury phosphate. None proved appreciably better than the Bordeaux spray. In repeated tests with very young seedlings good disease control was secured, but extensive plant bed experiments in 1932 and 1933 at locations in 5 States gave mostly negative results. The disease usually developed after the plants had attained some size and adequate coverage was practically impossible. Spraying the larger plants may cause loss by reducing their ability to stand transplanting. Control of the disease was secured in plant beds heated at night to maintain 70° F. or above during the critical period. This control was obtained without modifying humidity relations or day temperature. It is believed that this method of protection would be less effective with very young plants.

*Winter Injury and Drought in Relation to Apple Root Rot (Xylaria mali).* J. S. COOLEY.

Winter injury is not uncommon in hillside apple orchards in Virginia and other eastern States. Some of this injury, which is usually on the larger roots or about the collar of the tree, occurred in the winter of 1932-33 and some in former years. It is much more severe on trees growing on the lower slope of the hill than on those near the crest. It is probable that poor aeration predisposes to injury by even moderate cold. The excessive dryness of the subsoil since the drought may have so changed the physical properties of the subsoil as to alter its seepage conditions. Black root rot (*Xylaria mali*) is much more prevalent on the lower slope of certain of these hillside orchards than on the higher and better-drained land, where winter injury is less pronounced. An examination of the trees affected with root rot indicates that winter injury may be a very important predisposing factor to infection by *Xylaria* root rot. Drought, as a contributing factor to the incidence of root rot, is attested by greater prevalence of disease on those trees located where they suffered from drought. The question of disease distribution, however, needs to be considered in a study of predisposing conditions.

*Fungicidal Control of Gymnosporangium juniperi-virginianae and Related Species.*  
IVAN H. CROWELL.

For the past 3 years experiments at the Arnold Arboretum of Harvard University have been carried out on fungicidal control of various species of *Gymnosporangium*, both with respect to red cedars and with pomaceous susceptibles. Of the many fungicides tested, one has given satisfactory results—a special form of pure colloidal sulphur sup-

plied through the courtesy of a Boston chemical firm. This material has given essentially complete protection to pomaceous plants, as determined from hundred of controlled experiments carried out on a small scale. Direct attempts, with some interesting results in hand, to control various species of *Gymnosporangium* on red cedars are in progress. Of special importance is the possibility of controlling cedar-apple rust without having recourse to eradication of the red cedar.

*Relative Susceptibility of the Species of Malus to Gymnosporangium juniperi-virginianae.*

IVAN H. CROWELL.

Opportunity has been afforded in the Arnold Arboretum of Harvard University for testing the susceptibility of all but 2 or 3 of the known species of *Malus*. The results show a range from high susceptibility to complete immunity. All species of the section *Chloromeles* of the genus *Malus* are susceptible. These species are native to eastern North America. Besides these, *M. fusca* of western North America is susceptible, though more resistant than the species of the section *Chloromeles*; the same is true of *M. sylvestris* of Europe. All other species (native to Eurasia) are immune. Complete series of tests were made with inoculum from Alabama, Kentucky, Massachusetts, Michigan, Mississippi, Nebraska, New York, and West Virginia. Interesting differences were noted in the aggressiveness of the pathogen from these areas—an indication of biological strains. It was also observed that the more highly susceptible species of *Malus* are liable to infection over a longer period than the less susceptible species.

*Twig Blight (Hypomyces ipomoeae) of the American Bladder Nut.* W. H. DAVIS.

Tips of dead twigs on bladder nut, *Staphylea trifolia*, were spotted with rose-colored spore masses bearing microconidia and macroconidia that were fusarial in appearance. This fungus is *Hypomyces ipomoeae*, for which bladder nut seems to be a new host.

The blight first appeared on young twigs about July 15th, and in wet, warm weather was accompanied by sporulation. From 10 to 50 per cent of the root sprouts may be killed and the main stem may be girdled and killed.

Infection was obtained by employing inoculum composed of viable conidia and ascospores. Wounding was not necessary for penetration and infection, but it aided. When leaves, old stems, maturing twigs, buds, and meristematic tissues were inoculated, only buds and meristem showed infection. Both conidial stages and the ascogenous stage formed in the host. Perithecia formed in the dead bark during late October and contained viable ascospores, which germinated best during the following May. The perfect stage was cultured in green, disinfected stems of the host, which were inoculated and incubated under laboratory conditions.

Hypphae remained viable in the pith and nodes of stems during the winters beginning with 1931 and with 1932.

*Cortical Parasitism of Conifer-Seedling Roots in Pure Culture by Mycorrhizal and Nonmycorrhizal Fungi.* K. D. DOAK.

A fungus, resembling *Rhizoctonia silvestris*, isolated from roots of *Pinus taeda*, not only formed the mantle and Hartig network characteristic of ectotrophic mycorrhizae on *P. taeda*, *P. resinosa*, *P. strobus*, *P. rigida*, and *P. radiata*, but also infected the cortex of mother roots. Although the latter type of infection was extensive, roots of *P. taeda* were not killed after 18 months in culture.

Two physiologic strains of *Armillaria mellea* grown with *Pinus strobus* and *P. rigida* infected the cortices of short roots and mother roots. Rhizomorph-like groups



of hyphae, penetrating the cortex, did not branch into individual cells or incite conditions indicating injury other than resin accumulation in cells near points of entrance. Seedlings of *P. strobus*, grown with one strain for 18 months, were not killed but showed more yellowing of fascicled needles than did the controls. Cork production at the points of emergence of lateral roots occurred in control cultures as well as with both strains of the fungus. One case of casual infection of a cork mass was found.

*Fungi that Produce Ectotrophic Mycorrhizae of Conifers.* K. D. DOAK.

In pure-culture syntheses the following combinations of fungi and coniferous seedlings gave typical ectotrophic mycorrhizae: *Boletus bicolor* with *Pinus rigida*; *B. granulatus* with *P. strobus* and *P. taeda*; *B. eximus* with *P. taeda*; *B. brevipes* with *P. rigida* and *P. taeda*; *B. chromapes* with *P. taeda*; *Boletinus pictis* with *P. strobus*, *P. taeda*, *P. resinosa* and *P. rigida*; *Cantharellus cibarius* with *P. taeda* and *P. strobus*; *Amanita muscaria* with *P. taeda* and *P. strobus*; *Russula lepida* with *P. rigida*, *P. taeda* and *P. strobus*; and *Scleroderma vulgare* with *P. strobus*. The mantle and Hartig network characterizing this form was demonstrated histologically in each case. The fungi were obtained in culture by isolations from the unexposed tissues of sporophores. Their growth was facilitated in the sand culture with sterile seedlings by use of 0.5 per cent dextrose in a mineral nutrient solution, and reisolations of the fungi from the synthesis cultures were successful in most cases.

*Pythium butleri* and *P. aphanidermatum*. CHARLES DRECHSLER.

Of nearly 100 cultures of *Pythium* isolated from various plants wherein the sporangia are often characteristically lobulate and the antheridium regularly consists of a swollen protuberant part broadly applied to the oogonium together with an unmodified portion of the supporting branch, 2 originating from diseased tobacco seedlings in Sumatra and 3 from affected sugar cane roots in Louisiana, apparently represent a different species from the others. The generally smaller zoosporangia here are derived from elements much less extensive, less swollen and less elaborately branched. The zoospores are smaller, their diameter, after encystment, averaging about  $9\mu$  instead of  $11\mu$ ; and their production is difficult to induce on artificial substrata. The sexual structures are smaller, the oogonial diameter averaging about  $22\mu$  instead of  $27\mu$ , the oospore diameter about  $17.5\mu$  instead of about  $22.5\mu$ . The smaller antheridium is much more frequently produced immediately adjacent to an oogonium, though here also an adjacent antheridium generally fertilizes some less directly connected oogonium. Of the 2 species thus differing not only in dimensions (which, besides, vary considerably among the several strains) but also in reproductive behavior, the smaller one appears appropriately designable as *P. aphanidermatum*, the larger one as *P. butleri*.

*Vascular Wilt and Root Rot of Pansies due to Aphanomyces sp.* CHARLES DRECHSLER.

Late in April, 1933, at Mt. Rainier, Maryland, pansies in beds previously planted with pansies several years in succession, developed symptoms of wilt, which during the following weeks became increasingly severe. At this stage neither the stems nor the well-developed root systems of affected plants showed any evidence of cortical decay or of other external injury, but the vascular cylinder throughout was discolored deep red or orange red, very much as in some vascular wilt infections due to *Fusarium* spp. By the middle of May a general decay involved, in many plants, the base of the stems and the roots some distance downward, the aerial parts then collapsing, dying and drying up completely. In the earlier stages wilted plants revealed under microscopic examination numerous oospores immediately surrounding the discolored vascular cylinders

of infected roots. These oospores belonged to a species of *Aphanomyces* very readily and consistently obtained in pure culture from affected material. This species differs morphologically from *A. euteiches* from peas and does not show quite satisfactory agreement with the fungus obtained from the Centraalbureau voor Schimmelcultures at Baarn as *A. euteiches* P. F. 2.

*Spraying and Dusting of Tomatoes for Late Blight (Phytophthora infestans).* A. A. DUNLAP.

Copper-lime dust and 4-4-50 Bordeaux spray were found effective in controlling the late blight of tomatoes during the past season. Spray treatment gave larger yields of both ripe and green fruits than did the dust. Decay of the tomato fruits was almost completely controlled by spraying.

*Seed Treatment Studies of Spinach.* L. E. ERWIN and F. K. CRANDALL.

In greenhouse trials with 10 different chemicals used as seed treatments on various vegetable seeds it was found that the best control with spinach was obtained by the use of  $\text{CuSO}_4$  (sol.), red oxide of copper, and P.D.-7. Further trials showed that P.D.-7 gave better control in every case, both in combating the preemergence phase and the postemergence phase of the disease. P.D.-7 compared favorably with red oxide of copper as to adhesiveness. Trials in the field on spring and fall spinach with P.D.-7 and  $\text{CuSO}_4$  (sol.) showed that the dust was just as effective as the  $\text{CuSO}_4$  solution. The P.D.-7 gave a uniform stand and the seedlings appeared above the ground 2 or 3 days earlier than in the case of the  $\text{CuSO}_4$  solution. Further trials with late fall spinach showed that P.D.-7 and red oxide of copper gave a vigorous and uniform stand, while the  $\text{CuSO}_4$  solution showed a very uneven stand, and the damping off organism killed many of the plants after they appeared aboveground.

*Comparing Soil Fungicide with Special Reference to Phymatotrichum omnivorum Root Rot.* WALTER N. EZEKIEL and J. J. TAUBENHAUS.

Laboratory tests have been devised for comparison of the fungicidal value of materials after they have penetrated the soil. In closed jars of soil, surface applications of pentachlorethane, tetrachlorethane, xylene, carbon disulfide, perchlorethylene, trichlorethylene, dichlorethylene, turpentine, and paradichlorobenzene at 100 ppm. of soil weight penetrated 135 mm. of moist soil and prevented *Phymatotrichum* growth. Chloroform, dichlormethane, benzene, kerosene, ethyl chloride, ether, and naphthalene were less effective; and New Improved Ceresan, DuBay 1153A, DuBay 971A, Semesan, formaldehyde, and ammonia, ineffective even at 2000 ppm.

Direct toxicity tests of materials, after passage through air and cotton plugs, only, proved pentachlorethane, tetrachlorethane, xylene, and ammonia most toxic to *Phymatotrichum*. Carbon disulfide, chloroform, perchlorethylene, trichlorethylene, dichlorethylene, ethylene dichloride, and carbon tetrachloride were somewhat less toxic. Dichlormethane, ether, New Improved Ceresan, DuBay 1153A, paradichlorobenzene, formaldehyde, acetone, and benzene were still less toxic; and naphthalene, ethyl chloride, hexachlorethane, and alpha naphthol not toxic within the limits of the tests.

In preliminary field tests, tetrachlorethane injected 6 inches into the soil had, within 15 days, killed *Phymatotrichum* on infected cotton roots to at least 24 inches deep. Field tests with chlorinated hydrocarbons and xylene are being continued to determine their practical value in eradication of root rot.

*Soil Treatment with Mercurials for Control of Potato Scab.* C. W. FRUTCHEY and J. H. MUNCIE.

In 3 years' trials in the field and greenhouse, mercuric oxide, mercurous chloride and organic mercury, DuBay 965H, applied in the row before planting, failed to control soil-borne potato scab. Soils in these fields gave pH readings of 6.8–7.2. Materials were applied at 10, 20, and 10 pounds per acre, respectively. Of the 3 materials DuBay 965H showed a slight decrease of scabby tubers in only 1 test, while mercuric oxide and mercurous chloride increased the percentage of scabby tubers as well as area of tuber infection in every case. Applications of mercuric oxide and mercurous chloride decreased the ratio of soil bacteria and fungi to *Actinomyces* under controlled low-moisture conditions. Under high moisture conditions the ratio was increased. Several strains of *Actinomyces scabies* grew well in saturated solutions of mercuric oxide and mercurous chloride in tyrosinate liquid media. No correlation was found between tolerance to mercurials and virulence of *A. scabies*.

*A Leaf Nematode Disease of Begonia.* D. L. GILL.

The leaf nematode disease of begonia, caused by *Aphelenchoides fragariae*, is characterized by brownish discolorations on the under sides of the leaves. The disease is serious on the semituberous types of begonia grown under glass. Fibrous-root begonias are less susceptible. Migration of the nematodes occurs only when the plants are wet. The nematodes enter and emerge from the leaves through the stomata, are capable of living at least 81 days in detached leaves, and can exist in infested soil at least 6 weeks. If healthy and diseased leaves are maintained in direct contact the healthy individuals remain free from infection as long as the foliage is kept dry. Rapid spread takes place under the same conditions when the leaves are sprinkled. The thermal death point of the nematodes in the leaf tissue (47° C. for 5 minutes) is harmful to the plants. Fifty-six chemicals were tested for control without success. The disease, however, can be controlled by using healthy leaves for cuttings, avoiding infested soil, isolating diseased plants, removing infected leaves, spacing to avoid direct contact of plants, and keeping the foliage dry.

*Sulphur Vaporization with Rupprecht's Sulfurator in Greenhouse-Crop Prophylaxis.* E. F. GUBA.

This simple but remarkable invention for generating sulphur vapors from a specially constructed charcoal furnace promises to play a great rôle in crop-disease and insect control and to displace the use of other forms of sulphur fungicides and insecticides in greenhouse use.

In the past many commercial types of apparatus for vaporizing sulphur, notably Campbell's Sulfur Vaporizer, Rupprecht's Rota-Generator, Monro's Sulfur Generator, the Sulphur Fumigator of Cooperative Tuinbouw Audoopvereniging, and simple home-made units comprising stove and container, have been used, but none approaches Rupprecht's Sulfurator in safety and practical use.

*Control of the Narcissus Leaf-Scorch under Long Island Conditions.* F. A. HAASIS.

Narcissus leaf-scorch (*Stagonospora curtisii*), although not a major disease in narcissus-bulb production, causes considerable reduction in crop yields. With certain exceptions, the varieties belonging to the *Poeticus*, *Leedsii*, and *Barrii* groups are more severely affected. Moderate temperature, precipitation, and an abundance of primary infections giving rise to secondary inoculum are important factors in promoting

epiphytotics. Soaking the bulbs in water for 1 hour, followed by a 30-minute dip in mercuric chloride 1-750 or formaldehyde 1-120 prior to planting, will greatly reduce primary infections. Secondary infections can be controlled with timely applications of copper fungicides, 4-4-50 Bordeaux spray or 20-80 copper-lime dust. The foliage should be protected from the first week in May until the third week in June. In general, this can be accomplished with from 4 to 5 applications. It is important, however, that the applications precede periods of moderate to heavy precipitation.

*Toxic Action of Trichoderma on Rhizoctonia and other Soil Fungi.* C. M. HAENSELER and M. C. ALLEN.

Trichoderma added to a soil heavily infested with Rhizoctonia reduced seed decay and damping off on cucumbers and peas from 67 and 63 per cent to 12 and 38 per cent, respectively. With a Pythium-infested soil the reductions were from 69 and 77 per cent to 12 and 47 per cent, respectively. A liquid nutrient medium in which Trichoderma had grown for 5 days, sterilized by passing through a Seitz filter, proved to be lethal to Rhizoctonia and Pythium. It was necessary to dilute this toxic filtrate to approximately  $\frac{1}{2}$  its concentration with water, fresh nutrient, or "deactivated" filtrate before growth could take place. The toxic properties of the filtrate were destroyed by heating for 10 minutes at 80° C., by aging in cotton plugged test tubes or flasks for 10 days, or by bubbling oxygen through the medium for 5 minutes. Preliminary attempts to concentrate the toxic principle by precipitation with alcohol were unsuccessful.

The results indicate that the occurrence of Trichoderma in the soil may have an antagonistic effect upon Rhizoctonia and possibly other soil-borne plant pathogens, and that this antagonism may in part be due to toxic materials elaborated by the Trichoderma.

*Preliminary Note on the Relation of Mycorrhizae to Dry-Weight Increase in Pinus strobus.* A. B. HATCH.

Dry weights of 3-month seedlings grown from weighed seeds in pot cultures (27 seedlings each) using soils of different fertilities, were examined for simple correlation with numbers of mycorrhizae. Seedlings in 5 out of 37 pots yielded significant values for  $r$  (Fisher's table of  $t$ )—3 positive and 2 negative. In one case duplicate pots gave significant correlations of opposite sign, revealing the fortuitous character of significant values of  $r$  for this material. Accordingly, the relationship was apparently casual. It seems permissible, therefore, to predict that mycorrhizae were not detrimental.

A similar prediction concerning their possible beneficial rôle is not justified, since masking of beneficial effects could occur, in that: (1) low internal nutrient concentrations are seemingly responsible for mycotrophism in *Pinus*. Therefore, seedlings that initially develop mycorrhizae possess low, internal nutrient concentrations, and those that remain nonmycorrhizal possess high ones. (2) The beneficial theory postulates superior absorptive efficiency for mycorrhizae, hence seedlings differing in nutrient reserves at germination should tend toward uniformity when exposed to mycorrhizal inocula. Apparently, this may occur, since seedlings grown in soil cultures have lower coefficients of variation than sand-culture seedlings, where mycorrhizal inocula are lacking.

*Effect of Air Temperature on Tobacco Ring-Spot Infection.* R. G. HENDERSON.

Studies conducted in the greenhouse and in incubators indicate that the development of ring-spot symptoms, following artificial inoculation, is influenced by the air temperature in which the plants are grown. At temperatures above 93° F. symptoms did

not develop, but at 80–85° symptoms developed in 4–5 days. Plants with primary lesions on the inoculated leaves, when transferred from a low temperature (80–85°) to a high temperature (above 93°), did not develop further symptoms. On the other hand, plants, held at a high temperature for several days and on which no symptoms had appeared, developed primary lesions on being transferred to a low temperature.

*Experiments on the Control of Downy Mildew of Tobacco.* R. G. HENDERSON.

Several fungicides were used on plant beds under field conditions last spring in an attempt to find one that might be effectively used in controlling tobacco downy mildew (*Peronospora hyoscyami*). Of the fungicides used in the experiment, calcium sulphide (Cal-Mo-Sul) alone gave results approaching commercial control. Bordeaux gave only slight control. Similar results have been secured by experiments conducted under greenhouse conditions. It seems necessary, however, to make more frequent applications in the greenhouse than in the field in order to get satisfactory control. Although the available data are not conclusive, calcium sulphide apparently is a promising fungicide for downy-mildew control in tobacco-plant beds. Where frequent applications of calcium sulphide have been made on small seedlings in the greenhouse, there has been a noticeable stunting of plant growth.

*The Origin of Roots Stimulated by Hairy-Root Bacteria in Apple Stems.* E. M. HILDEBRAND.

The disease of nursery apple trees known as “infectious hairy root” is of interest to botanists generally because the causal organism, *Phytomonas rhizogenes*, stimulates the formation of roots. Hairy roots originate in the first stages of the disease over a period of approximately 4 weeks after inoculation. They begin in the callus-disease tissue, elaborated by the host, due to the combined effects of wounding and the presence of the bacteria. Wounding alone apparently stimulates the ray cells of the xylem and cells of all the living tissues outside the woody cylinder, except the epidermis (periderm), to multiplication, which may soon cease with the reestablishment of cambial continuity. The presence of the bacteria, however, largely prevents this reestablishment and there is further proliferation and root formation. The first and second weeks are marked by cell multiplication over practically the entire face of the wound. When present, bacteria, crushed cells, and foreign matter may become entirely surrounded. The resulting islands appear as somewhat circular areas of hyperplasia. Beginning late in the second week meristematic localities appear in the elaborated tissues from which the roots later develop quite independently of the deeper normal tissues.

*Masked Strain of Tobacco-Mosaic Virus.* F. O. HOLMES.

A study has been made of a strain of tobacco-mosaic virus, the effects of which are masked in *Nicotiana tabacum*. The masked strain and the ordinary strain of tobacco-mosaic virus are alike in their responses to heating, storage, and dilution and in their host range. In all necrotic-type hosts primary lesions of the strains are indistinguishable in appearance. In hosts in which systemic necrosis or intense yellowing are produced by the ordinary strain the symptoms of the masked strain are less severe. Hosts in which mottling is produced by the ordinary strain act as symptomless carriers of the masked strain. In tobacco both the masked and the ordinary strain of tobacco-mosaic virus cause abnormal starch retention in recently affected leaf tissues. This effect can be made conspicuous by removal of chlorophyll, and subsequent staining with

iodine. The areas of young leaves thus shown to be affected differ for the 2 strains, systemic spread of virus being less extensive in plants infected with the masked strain.

*Zinc Oxide as a Seed and Soil Treatment for Damping Off.* JAMES G. HORSFALL.

The red copper oxide seed treatment recently proposed (N. Y. S. Agr. Exp. Sta., Bul. 615) for damping off increases stands markedly and widely, particularly in field crops. Sometimes, however, it is not wholly satisfactory for prevent post-emergence damping off in greenhouses caused by *Pythium ultimum*. A search was instituted to discover a chemical having persistent fungicidal properties applicable to the soil surface without producing undue plant injury. Soluble zinc compounds, having been used as wood preservatives and for fire-blight control, were tried and found to be fungicidal but excessively toxic to the plants. Moreover, their fungicidal effect was transitory.

Zinc oxide, under test since January, 1931, is insoluble in water, therefore persistent, is relatively noninjurious when applied before emergence, and is satisfactorily fungicidal. Being insoluble, it penetrates poorly into soil; hence is inefficient for increasing stands, a difficulty easily overcome by mixing it with the covering soil or by using it with a seed treatment. Zinc oxide itself is an effective seed treatment, sometimes appearing superior to red copper oxide. Used at 10 to 20 grams per square foot, it has been commercially successful in greenhouses in several New York State localities. It has been applied to transplanted seedlings but occasionally injury results.

*Correlation Between Rough-Hairy Pubescence in Soybeans and Freedom from Injury by Empoasca fabae.* E. A. HOLLOWELL and H. W. JOHNSON.

To determine if the field-observed freedom of rough-hairy varieties of soybeans from the injury caused by the potato leaf hopper is correlated with the occurrence of rough-hairy pubescence in material of known genetic constitution, the writers obtained from C. M. Woodworth of the Illinois Agricultural Experiment Station seed of 29 glabrous  $F_1$  plants from a cross between Illini (rough-hairy) and a dominant glabrous type of soybean. Of the  $F_1$  progenies from these plants 14 were homozygous-glabrous and 15 were heterozygous, segregating in a ratio of approximately 3 glabrous to 1 rough-hairy. Of the  $F_2$  progenies, 47 were homozygous-glabrous, 32 heterozygous, and 29 homozygous rough-hairy. Of the  $F_3$  progenies, 32 were homozygous-glabrous, 32 heterozygous, and 9 homozygous rough-hairy. In the 3 generations grown the glabrous individuals (total number 8570) of both the homozygous-glabrous and heterozygous progenies were all heavily infested with *Empoasca*, severely stunted in growth, and had curled leaves with yellowed, necrotic margins. The rough-hairy individuals (total number 6755), on the other hand, were almost entirely free from *Empoasca*, grew vigorously, and their leaves showed no symptoms of leaf-hopper injury. It is concluded that in soybeans, at least with the material under test, freedom from injury by the potato leaf hopper is correlated with the occurrence of rough-hairy pubescence.

*Partial Recovery and Immunity of Virus-Diseased Abutilon.* JOHN Y. KEUR.

It has been noted frequently that variegated Abutilon plants may recover entirely or partially from the effects of the virus. In case of partial recovery, green-leaved branches develop on variegated virus-diseased plants. Baur claimed that such branches, developing on *Abutilon thompsonii*, were immune. The following results were obtained with green branches of 5 variegated Abutilon species: (1) The virus is absent in such branches as demonstrated by the inability of scions to infect susceptible green Abutilon clons. (2) The virus does not pass through such green branches in sufficient quantity to produce variegated symptoms in susceptible green scions grafted on the green

branches. (3) These green branches are not actually immune. Green clons propagated from them can be reinfected not only by scions of other variegated Abutilon clons, but also by scions of their own variegated clons.

*Tobacco and Aucuba-Mosaic Infections by Single Units of Virus.* L. O. KUNKEL.

When two samples of tobacco juice containing the viruses of aucuba and tobacco mosaics, respectively, are mixed in equal or other proportions up to one part of either in 30 parts of a mixture and used to inoculate mature leaves of *Nicotiana langsdorffii* plants by the needle-puncture method, circular necrotic lesions of uniform size and color develop around those punctures at which infection occurs. By subinoculations from these leaves to plants of *Nicotiana glauca* it is possible to show whether any given lesion contains tobacco mosaic virus, aucuba mosaic virus, or a mixture of the two. By making use of this technique in the study of a large number of infections, it has been shown that needle-puncture inoculations from mixtures of the 2 viruses give lesions containing either one or the other, but seldom both. Since each virus is readily transmitted by the needle-puncture method, the data obtained bring evidence that, except in a small percentage of cases, infection results from a single virus unit. The method is, therefore, suitable for the isolation of pure cultures derived from single units of either virus. The lesions are comparable to bacterial colonies isolated by the Koch plate method.

*Experimental Production of Crown Gall on Opuntia.* MICHAEL LEVINE.

Attempts of the writer to induce the crown gall disease on *Opuntia* species have up to the present been unsuccessful. *Opuntia Keyensis* after seven years produced large, well developed galls after inoculation with *B. tumefaciens*. The growth appeared to form four months after the inoculation of young well-rooted joints. One year after inoculation the galls attained the size of a black walnut, and at present show no tendency to ageing or necrosis. Microscopical studies of this gall show a disoriented conglomeration of cells consisting of fibro-vascular elements, clusters of embryonic cells and parenchymatous tissue surrounded by fragments of epidermis and bark. There are also a number of definitely organized structures which to all appearances are embryonic roots developing in the periphery of the crown gall. Many host cells, as well as crown-gall cells, contain aggregates of crystals while only the host tissue contains mucilage or slime cells. The majority of *Opuntias* so far studied appear to lack the protecting mechanism of forming overgrowths common to a large number of dicotyledonous plants in that the introduction of the tumor-producing organism results in injury and death of the tissue. Laboratory Division, Montefiore Hospital, N. Y. C.

*The Production of an Apparently New Variety of Puccinia graminis by Hybridization on Barberry.* M. N. LEVINE, RALPH U. COTTER and E. C. STAKMAN.

In an effort to determine the extent to which new parasitic strains of *Puccinia graminis* may result from hybridization between existing strains on barberries in nature, artificial crosses were made in the greenhouse between certain physiologic forms of *P. graminis tritici* and *P. graminis secalis*. As a result there were obtained lines that attack certain varieties of barley normally but not the other cereals. As the new lines seem restricted to barley, they could properly be designated as a variety. The following crosses were made: *P. graminis secalis* 7  $\times$  *P. graminis tritici* 21 and *P. graminis secalis* 7  $\times$  *P. graminis tritici* 101. In both cases the progeny were similar. There is some evidence also that *P. graminis tritici* 34  $\times$  *P. graminis agrostidis* produced a rust

similar to the above.—(Division of Barberry Eradication and Division of Cereal Crops and Diseases, U. S. Department of Agriculture, in cooperation with the Minnesota Agricultural Experiment Station.)

*Some Aspects of the Pathology of Forest Trees and Forest Products in Middle Europe and Scandinavia in 1933.* R. M. LINDGREN.

*Nutritional Relationship in the Apple-Rust Fungus.* J. C. LIU.

In apple rust the aecium began its formation about 40 days after inoculation, although the epidermis was not ruptured until 20 days later. Toward the end of the first month after inoculation the spermogonium is in full development but the mycelium can hardly be distinguished under the microscope. In 30–40-day-old lesions, however, the mycelium appears quite vigorous. After the formation of the aecia the mycelium is again indistinct. Such behavior of the mycelium is explained as follows: The spermogonium, being a large organ, with multiple functions to perform, naturally requires an abundance of food, which can be supplied only by the mycelium. Due to this drain, the mycelium becomes depleted. After the spermogonium has completed its development and activities, the demand for food decreases. Consequently, the mycelium becomes replenished and is in a condition to initiate the aecium. Development of the aecium means a further food requirement. The food supply is diverted from the spermogonium to the newly-formed aecium. Consequently, the former dies of starvation and its exudation ceases.

*Inheritance of Resistance to Powdery Mildew, Erysiphe graminis tritici, in Wheat.*  
E. B. MAINS.

The inheritance of the resistance of Norka C. I. 4377 to physiologic form 1 of *Erysiphe graminis tritici* has been studied in crosses between Norka and the susceptible varieties Webster, Ceres, Chinese, Kota, Malakoff, Reliance, and Warden. In these crosses resistance was inherited as a simple dominant factor, the segregation in the  $F_2$  being approximately 3 resistant : 1 susceptible. Resistance to mildew was inherited independently of the resistance of Norka to physiologic form 3 of leaf rust, *Puccinia rubigo-vera tritici*. The resistance of Red Fern to mildew has been found to be inherited also as a simple dominant Mendelian factor. The resistance of Hope C. I. 8178, in crosses with the susceptible varieties Chinese, Marquis, Michigan Amber, and Reliance, was inherited as a simple, recessive Mendelian factor, the ratio in the  $F_2$  being approximately 1 resistant : 3 susceptible. The resistance of Sonora and the resistance of Michigan Amber 29–1–1 in crosses with Chinese were each also inherited as a simple recessive factor.

*Relative Infectivity of Mosaic Virus Extracted from Various Parts of Sugarcane.*  
JULIUS MATZ.

Using methods of inoculation found superior to previous methods for insuring infection with extracted juice (Jour. Agr. Res. 46 (9): 821, 1933) tests were made to find whether, in an infected plant, there might exist specific regions in which the activity of the virus is, at least partly, normally inhibited or inactivated. Results to date show that in individual shoots juices from infected expanded lamellae of young and mature green leaves, midribs, leaf sheaths, and true stems, separately, are equally infectious to a high degree (80–100 per cent), whereas the juice from the rolled inner white and brittle portion of the leaf bases in the lower region of the pseudostem, from the same individual shoots in parallel series of inoculations made at the same time



was comparatively low in infectivity (0-50 per cent). The symptoms and their time of appearance in all tests showed no deviation in any noticeable manner from normal virulent sugarcane mosaic, indicating no qualitative change but suggesting a quantitative reduction of virus in the juice expressed from the inner white false stem as compared with the juice from the other sources. However, the addition of equal volumes of healthy false-stem juice to virulent green-foliage juice after extraction did not result in low infectivity, nor was there any reduction in infectivity when infectious green-foliage juice was diluted with an equal volume of healthy green-foliage juice.

*Susceptibility of Treated and Untreated Turf to Brownpatch and Dollarspot.* H. F. A. NORTH and L. E. ERWIN.

During the progress of experiments carried on by the Rhode Island Experiment Station, many of the plats of fine turf have been heavily damaged by brownpatch (*Rhizoctonia solani*) or by dollarspot (*Rhizoctonia* spp.). The average percentage of area affected by these diseases in 1931 to 1933 has been computed for 15 plats of colonial bent, *Agrostis tenuis*, 19 plats of velvet bent, *Agrostis canina*, and 19 plats of creeping bent, *Agrostis palustris*. Marked seasonal and varietal variations in disease have been the rule, but in general, grasses have tended to continue susceptible to one or both diseases from year to year. Colonial bent plats have been susceptible to brownpatch but highly resistant to dollarspot. Most of the strains of velvet bent appear rather resistant to brownpatch but susceptible to dollarspot. Creeping bents, though more variable than the two other species have resembled velvet bent in susceptibility. Good control of these diseases has been obtained by the use of bichloride of mercury 1/3, and calomel 2/3, but much depends upon local weather conditions. A test of trade products advertised for the control of brownpatch shows that few are more effective than the mixture cited above and a number that are much less useful.

*Incubation Period of Pea Mosaic in Macrosiphum pisi.* H. T. OSBORN.

The pea aphid has been used as a vector in the transmission of pea mosaic to the broad bean, garden pea, sweet pea, and Canada field pea. Because of the ease with which it may be grown, the broad bean was used in most experiments. It has been found that the pea aphid, after feeding on infected plants, acquires the ability to infect healthy plants and that this ability is retained throughout the active feeding life of the insect. By exposing a succession of healthy plants to colonies that were allowed to feed for a short period on diseased plants, an incubation or noninfective period has been shown to exist. In these experiments the period varied from 12 to 28 hours. Mechanical transmission of pea mosaic to the broad bean is difficult.

*Rosette of Blackberries and Dewberries.* A. G. PLAKIDAS.

The rosette, which is apparently identical with the "double blossom" disease of the Southeastern States, is the most serious disease of blackberries and dewberries in Louisiana. Although the double blossom disease has been generally attributed to *Fusisporium rubi*, studies made in Louisiana indicate that the causal agent of the rosette is a species of *Cercospora*. The disease was repeatedly produced by inoculation with pure cultures of this fungus. Natural infection takes place on the primocanes in the spring, but the symptoms do not appear until the following spring when these canes resume growth. At that time the infected buds give rise to witches' brooms, and to abnormal blossoms that do not set fruit. The period during which infection takes place is from about the middle of March, when the spores begin to appear on the diseased blossoms, to about the middle of June. Spores are found in abundance on the withered blossoms until the

last part of August, but very little infection occurs after the middle of June. Spraying the primocanes with 4-4-50 Bordeaux every 10 days from the first of April to the first of June gave practically complete control (about 98%) of the disease.

*Sweet-potato Ring Rot Caused by Pythium ultimum.* R. F. POOLE.

A specific soft decay of sweet potato, ring-like in character, working from one side of the root to another without involving all tissues, is found to be due to *Pythium ultimum* and not to *Nectria ipomoeae* nor to *Rhizopus nigricans*, which were for some time attributed causes.

The fungus works intercellularly and intracellularly; produces anylase, pectinase, and protease but, apparently, no diastase. Rapid decay occurs in 36 to 48 hours and complete rings develop in 3 days at a temperature of 20° C. in an atmosphere saturated with moisture.

Ring rot developed after inoculation in all commercial varieties of sweet potatoes. The Jersey and Nancy Hall varieties were apparently most susceptible.

The disease was severe on sweet potatoes harvested after rains maintained high soil moisture conditions in late autumn. It was of no importance on sweet potatoes harvested prior to the rains.

*Apple Target Canker, Measles, and Rough Bark.* JOHN W. ROBERTS.

A bacterium, probably *Pseudomonas papulans*, which Rose described as the cause of apple blister-spot and probably the cause of apple measles in Missouri, has been isolated from the papulate early stage of apple target canker. Although inoculation of "Delicious" apple twigs has been followed by lesions typical of the early stages of the disease and the bacterium has been reisolated from these lesions, occurrence of the disease on noninoculated checks made the results inconclusive. Isolation of what appears to be the same organism from the papulate early stages of the rough-bark disease of the Yellow Newtown apple throws doubt on a former assertion of the writer that the rough-bark disease is caused by *Phomopsis mali*. Apple measles, target canker, and the rough-bark disease may prove to be identical, since their early stages are alike and what appears to be the same species of bacterium has been isolated from the early stages of all of them. They may also be identical with the apple diseases with which Lacy and Dowson in England and Hopkins in South Africa found organisms similar to, if not identical with, *Ps. papulans* to be associated.

*Studies on Barley Smut in 1933.* MABEL L. RUTTLE (MRS. NEBEL).

Further studies on the barley-smut types described by the writer in 1932 have been made. Seed inoculations with *Ustilago hordei* and Types 2, 3, and 4 and blossom inoculations with *U. nuda* have produced the respective types, with certain exceptions. The description of Type 6, only, has been altered. It is now regarded as having a loose head, and chlamydospores, olivaceous-brown in mass, which germinate by promycelia and sporidia rather than by germ tubes, as is more usual with spores of this color. Type 4 corresponds closely to the descriptions of *U. nigra* Tapke (1932) and *U. medians* Biedenkopf (1895).

Crosses between monosporidial lines of *Ustilago hordei* (covered head) and Type 3 (loose head) have been made. The  $F_1$  smutted heads resembled those of Type 3, but are possibly slightly more covered. The chlamydospores are smooth, like those of the parents, and germinate readily by promycelia that cut off sporidia. The sporidia culture easily. Seed inoculated with bisporidial cultures of *U. hordei* and Type 3 gave plants infected with the respective parental types.

Tennessee Winter barley was resistant to the form of *Ustilago nuda* collected on Featherston barley and susceptible to *U. hordei* and Type 4. Featherston barley was not infected with one collection of Type 4 but was infected by *U. hordei*, *U. nuda*, and Types 2, 3, and another collection of Type 4. Alpha barley was susceptible to all collections of the different types so far studied.

*Components of Potato Mild Mosaic.* E. S. SCHULTZ, R. BONDE and W. P. RALEIGH.

Mild mosaic, manifested by irregular light green spots and by slight wrinkling on Green Mountain potatoes, has been found to consist of 2 components. These components are a latent one that is masked in the variety Green Mountain as well as in many other varieties, including a Green Mountain seedling; and a second component, manifested on a seedling potato resistant to the latent component and on a latent-free Green Mountain seedling by light green and slightly rugose leaves. The second component has been separated from the latent component by means of aphids, which transmit the second but not the latent component. It also has been separated by means of a seedling potato, which takes the second but is resistant to the latent component. Inoculating healthy Green Mountain seedlings with both of these components produced typical mild mosaic. Moreover, inoculating Green Mountains harboring the latent component with the second component yielded mild mosaic. The reaction of latent-free Green Mountains to the second component has not been determined.

*Intercellular Relative Humidity in Relation to Fire-Blight Resistance in Apple and Pear.* LUTHER SHAW.

Resistance to *Erwinia amylovora* (*Bacillus amylovorus*) in apple and pear tissues varies greatly as the environment, particularly the amount of moisture, is varied. Studies were made to determine if these variations in resistance are related to differences in the relative humidity in the intercellular spaces of the host tissues. Measurements were made of growth of the blight pathogen on a variety of culture media in equilibrium with the following relative humidities: 99.9 (approximate), 99, 98, 97, 96, and 95 per cent. These humidities are approximately equivalent to osmotic pressures of 2, 12, 27, 41, 55, and 68 atmospheres, respectively. Maximum growth was obtained at 99.9 per cent relative humidity; at 99 per cent growth was reduced to approximately  $\frac{1}{2}$  of maximum; at 98 per cent approximately  $\frac{1}{10}$ ; and at 97, 96, and 95 per cent almost no growth occurred. Measurements were made of the relative humidity in the intercellular spaces and the relative fire-blight resistance of apple and pear tissues at different soil moistures and atmospheric humidities. Results of these tests showed a direct relation between increased fire-blight resistance and lowered relative humidity in the intercellular spaces of the tissues. Ninety-seven per cent relative humidity in the intercellular spaces was associated with complete resistance to infection and 100 per cent with maximum susceptibility.

*Persistence of Heart-Rotting Fungi in Girdled Trees.* PERLEY SPAULDING.

Among various features that have marked recent developments in forest pathology is the fate of heart-rotting fungi when their host trees are killed by girdling. Examination of 1,768 girdled trees, principally red maple, sugar maple, yellow birch, beech, and paper birch that have been girdled from 2 to 5 years has shown 80 bearing living sporophores of 5 different species of heart-rotting fungi, which certainly were established in the interior of the trunks before the girdling. This is a large enough number to serve as infection sources for the young stand. Older girdled trees will have to be examined to learn how long these fungi may persist in the dead trunks. In another

5 years most of them will be uprooted and fallen over. This will quite certainly result in the dying out of some of the fungi. However, if the snags fall in such a way that they can still soak moisture from the ground in any quantity the fungi will continue to fruit. But a relatively small percentage of them is likely to do this. Girdling or felling of infected trees will not result in a complete killing of these fungi, if there is no further disposal of the down stuff. Both will greatly reduce the number of living fungi and probably will almost eliminate them before a second stand of young trees reaches a susceptible age.

*The Pathogenicity and Cytology of Urocystis occulta.* E. C. STAKMAN, M. B. MOORE and R. C. CASSELL.

Inoculated rye seed was planted in soil maintained at the temperatures given, until plants emerged. The following percentages of smutted plants resulted: at 14° C., 47 per cent; 19° C., 35 per cent; 24° C., 24 per cent; 29° C., 0 per cent. In cooperation with Minnesota agronomists, 139 selfed lines of rye were tested for resistance. They ranged from highly resistant to completely susceptible. Eight varieties of rye were inoculated with 5 collections of smut, but there was no conclusive evidence of physiologic specialization. Opportunity for variation resulting from hybridization in *Urocystis occulta* appears more restricted than in those smuts whose sporidia become abjointed from the promycelium and bud extensively. Chlamydospores of *U. occulta* produce a promycelium on which are formed 2 to 6 uninucleate sporidia that apparently do not bud nor become abjointed. Sporidia on the same or different promycelia fuse in various ways, giving rise to binucleate hyphae of the dikaryophase, although occasionally sporidia appear to become binucleate without previous fusion. Vegetative hyphae in the host appear predominately binucleate, the diplophase resulting from nuclear fusions in the spore-mother cells.

*The Action of Trypsin on Tobacco-Mosaic Virus.* W. M. STANLEY.

The addition of a solution of crystalline trypsin to the nontreated juice of mosaic-diseased tobacco plants (*Nicotiana tabacum* var. Turkish) immediately rendered the juice practically noninfectious. Addition of trypsin to highly infectious purified solutions of tobacco-mosaic virus gave similar results when tested on *Phaseolus vulgaris* var. Early Golden Cluster, *N. glutinosa*, and *N. tabacum* var. Turkish. In many instances the infectivity of the virus was reduced to zero within 1 minute after the addition of the trypsin, yet no digestion of protein was detected by chemical analysis. The infectivity of the virus-trypsin solutions increased on dilution, standing, or moderate heating. The addition of globin and trypsinogen, which have no proteolytic activity, but have isoelectric points near that of trypsin, gave the same result as did the addition of trypsin. Pepsin, gelatin, and egg albumin, all of which have isoelectric points towards the acid side, were noneffective. The results indicate that the reduction in infectivity of the tobacco-mosaic virus is not due to the proteolytic activity of trypsin.

*Longevity of Sclerotia of Phymatotrichum omnivorum in Moist Soil in the Laboratory.* J. J. TAUBENHAUS and WALTER N. EZEKIEL.

*Phymatotrichum omnivorum* is known to survive in vegetative condition on roots of susceptible plants, and in dormant condition as sclerotia or strand sclerotia in the soil. Control of the disease by rotation with resistant crops depends on knowledge of the longevity in the soil of both stages of the fungus.

In October, 1929, sclerotia recovered from an infested carrot field at Temple, Texas, were placed in a number of small corked vials of moist Houston clay soil material and

stored in the laboratory. Each year 50 sclerotia have been tested for germination on moist filter paper in Petri dishes. Initial germination tests showed 68 per cent of the sclerotia viable. One year later, germination had decreased to 46 per cent, and in successive years to 28 per cent, 21 per cent, and 11 per cent, respectively. Some of the sclerotia that were still viable and germinated after 4 years' storage were placed on roots of normal cotton plants growing in glass jars. These sclerotia produced *Phymatotrichum* strands that attacked the cotton roots and eventually killed the plants, causing symptoms typical of *Phymatotrichum* root rot.

*Two New Diseases of the Texas Bluebell, Eustoma russellianum.* J. J. TAUBENHAUS and WALTER N. EZEKIEL.

Florists growing the Texas bluebell, *Eustoma russellianum*, have, during the last 5 years, experienced serious difficulty from diseases attacking this plant. Study showed that 2 distinct diseases were involved. One was a crown rot and damping off, caused by *Fusarium solani*. The second was a stem and leaf blight, which was proved to be caused by another fungus, *Sclerophoma eustomonis*, n. sp. Pure cultures of both organisms were isolated from infected material, the respective diseases reproduced artificially by inoculation of healthy plants, and the two organisms recovered again. Viable spores of both organisms have been found on insects from diseased plants. The fusariosis was controlled by soil sterilization with formaldehyde. The stem blight was controlled by spraying with 4-4-50 Bordeaux mixture plus casein as a spreader and nicotine sulphate as an insecticide.

*Further Studies on a Noninfectious Leaf-Deforming Principle from Mosaic Tomato Plants.* M. H. THORNTON and H. R. KRAYBILL.

When the expressed juice of mosaic tomato plants was autolyzed and the virus inactivated by boiling, the juice was found to contain the noninfectious, leaf-deforming principle described by Kraybill and Eckerson and by Kraybill, Brewer, Samson, and Gardner. The activity of the juice was not diminished by precipitation of the proteins with alcohol or acetone nor by extraction with ether. Numerous attempts to obtain active preparations from healthy plants were unsuccessful. Preparations from the fresh juice of tomato plants showing "fern-leaf" symptoms produced by inoculation with cucumber mosaic were inactive. Tomato plants showing marked symptoms produced by inoculation with the noninfectious leaf-deforming principle and tomato plants showing typical symptoms of mosaic were lower in reducing sugars, sucrose, starch, and hemicellulose and higher in total nitrogen and nitrate nitrogen than healthy plants.

*Susceptibility Reactions of Pinus sylvestris to Woodgate Rust.* R. P. TRUE.

Eosin-conduction experiments were made upon 1-, 2-, and 3-season material of infected and noninfected portions of susceptible twigs on August 18. These indicated that, while at first all the gall wood conducts, in the second season there appears a nonconducting core, which is later filled with discoloring depositions. By the third season this nonconducting, discolored core may either approach the cambium closely or occupy a comparatively small area at the center of the gall. In one case of the former the distal portion of the branch was dwarfed and dying. Following anatomical studies, infected and noninfected areas of susceptible twigs of corresponding ages were collected on September 20 and tested for cellulose, lignin, pectic compounds, starch, fats and oils, tannins, and resins and terpenes. Infected areas showed comparatively more of the reserve food substances and tannins. The number of resin canals was increased, but not disproportionately, to the increased volume of gall wood. Lignification

was suppressed to some degree by the presence of the fungus in the cambium. This tendency was especially pronounced near cambium areas that were being actively invaded.

*Purification of the Virus of Tobacco Mosaic.* CARL G. VINSON.

Juice from diseased plants may be increased in infective power more consistently by the addition of activated charcoal following the addition of Lloyd's reagent. A small amount of normal solution of aluminum sulphate produces a flocculent precipitate when added to the virus fraction obtained on decomposing the safranin precipitate of the virus with Lloyd's reagent. This precipitate packs sufficiently to allow complete decantation of the supernatant liquid. Very little activity has thus far been demonstrated in the supernatant liquid. The precipitate contains the infectious agent.

Magnesium sulphate also may be used to salt out the virus from the above-mentioned fraction, but a much greater quantity is required.

*The Stimulation of Fungus Spore Germination by Aqueous Plant Extracts.* FRANK WILCOXON and S. E. A. MCCALLAN.

In the course of toxicity studies difficulty is experienced in germinating in distilled water the spores of many species of fungi. Conidia of *Penicillium* sp. and *Neurospora sitophila* will not germinate at all, while the conidia of *Pestalotia stellata*, *Glomerella cingulata*, *Botrytis paeoniae*, and *Sclerotinia americana*, because of age or other factors, often give unsatisfactory germination. The addition of small quantities of tomato, orange, apple, or pear juice and aqueous extract of lily or gladiolus bulbs and dahlia or potato tubers resulted in nearly 100 per cent germination. Yeast extract and urine also stimulated. Extracts from *Coprinus micaceus* and *C. atramentarius* and tomato leaves did not stimulate. The stimulation afforded by standard nutrient solutions is greatly inferior. About 0.05 per cent of solids from tomato juice or yeast extract in the spore suspension will give rise to almost 100 per cent germination in *Penicillium* and *Neurospora*. The stimulating factor is heat-stable and dialyzable. Ascorbic acid (vitamin C) did not stimulate, nor did inositol when added to Bios II according to the method of Lash Miller. This factor appears to resemble the "Rhizopin B" of Nielsen.

*The Diurnal Cycle of Erysiphe polygoni.* CECIL E. YARWOOD.

Under field and greenhouse conditions the powdery mildew of red clover has shown a daily periodicity with respect to the division of the generative cell of the conidiophore, the maturation of the conidium, the discharge of the conidium, the conidial germinability, and the ability of the conidia to cause infection. The generative cell divides during the day and the distal of the 2 daughter cells develops within 2 days into the mature conidium. Typically, each conidiophore matures a single conidium per day, which is ordinarily liberated about noon. Spores taken from mildewed plants in the late morning and afternoon show a high germinative capacity, whereas spores taken from plants late at night or early in the morning germinate poorly. Inoculations made during the day result in a higher percentage of infection than those made at night. The diurnal cycle is no longer evident under conditions of continuous artificial light and is less marked during cloudy weather. In alternate artificial light and darkness (12 hours each) with temperature and humidity fairly constant, the diurnal cycle is similar to that under usual field and greenhouse conditions. *Erysiphe polygoni* on *Delphinium* spp., in the greenhouse, showed a similar diurnal cycle with respect to conidial formation and germinability.

(Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station.)

*Sclerotium Blight Destroys Winter Wheat in Gallatin County, Montana.* PAUL A. YOUNG.

Many sclerotia were found in the field on the straw of Turkey wheat, *Triticum aestivum*, and on dead stems of *Sisymbrium altissimum* in April, 1932 and 1933. Evidently these were sclerotia of *Sclerotium fulvum*. They were found only on land where *Sclerotium*-killed wheat leaves were collected then and during the preceding year. Cultures from the sclerotia on the overwintered wheat and mustard stems slowly produced sparse, hyaline hyphae and many sclerotia on cornmeal agar at 5° C. These turgid sclerotia were reddish brown and 700 to 1200 $\mu$  in diameter, with aggregate sclerotia 2200 $\mu$  in diameter. When dry, these sclerotia were black and 200 to 900 $\mu$  in diameter. Snow, melting from infested fields, revealed white, tan, or gray, unshriveled wheat leaves appressed to the soil. These leaves, killed by *Sclerotium fulvum*, contained many reddish brown to black sclerotia 200 to 500 $\mu$  in diameter. They did not produce a perfect stage in many experiments. Wheat, surviving winter blight, bore light-colored heads with shriveled grain. Forty varieties of wheat showed no resistance to natural infection by *Sclerotium fulvum*. Fall-sown wheat, emerging in the spring, escaped *Sclerotium* blight.

# VARIABILITY IN MONOCONIDIAL CULTURES OF *VENTURIA INAEQUALIS*<sup>1</sup>

D. H. PALMITER<sup>2</sup>

(Accepted for publication March 14, 1933)

Apple scab and its casual organism *Venturia inaequalis* (Cke.) Wint. have been under investigation at Wisconsin for some years, 10, 5, 18, and 6). The present paper reports a phase of these studies concerned with variation in the pathogen. This work (14) has dealt chiefly with types and magnitudes of variation. It is hoped that the results may facilitate further inquiry into the nature and causes of these phenomena.

## STUDIES OF PHYSIOLOGY

### Materials

Since 1929 monoconidial cultures have been isolated by the method described by Keitt (9) from scabby apples obtained from several localities in Wisconsin and from Oregon, Michigan, and New York. These isolates were then placed on malt-agar slants and transfers made at frequent intervals for stock cultures, which usually were kept in an ice-box at 8° C.<sup>3</sup> The malt agar used throughout the cultural studies, unless otherwise noted, consisted of 25 grams of Trommer's malt extract, 17 grams of agar, and one liter of distilled water. It was sterilized at 15 pounds' pressure for 20 minutes. All cultural comparisons were made with material incubated in dark control chambers.

These monoconidial isolations were numbered in the order in which they were isolated. Cultures from Wisconsin apples were Nos. 1-2 Dudley, 3-14 and 23 variety unknown, 15 Winter Banana, 16 Northwestern Greening, 17, 20 and 36 Fameuse, 21 McIntosh, 22 Delicious; from Oregon 18, 19, 33 Yellow Newtown, 34 seedling, 35 Northern Spy; from Michigan 24 Northern Spy, 25 Steel Red, 26 McIntosh; from New York 27 McIntosh, 28 Rome Beauty, 29 Fall Pippin, 30 Fameuse, 31 Northern Spy, and 32 Cortland.

### Colony Differences

*Colony Characters.* No two of the 36 isolations studied appeared exactly alike when grown under standardized conditions, yet duplicate cultures

<sup>1</sup> Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> The writer is particularly grateful to Dr. G. W. Keitt, under whose advice this work has been pursued.

<sup>3</sup> The temperature notations throughout this contribution are in degrees Centigrade.



could easily be recognized. Monoconidial reisolutions from the same culture were very uniform in colony characters. The cultures studied could be differentiated by such characters as: margin of colony, size of colony, color, and the amount of aerial mycelium. Many series of plate cultures were observed and the above characters were constant for a given culture under the same environment, with the exception of occasional sudden changes, which will be discussed later. Figure 1 shows some of the characteristic differences that distinguish the different cultures.

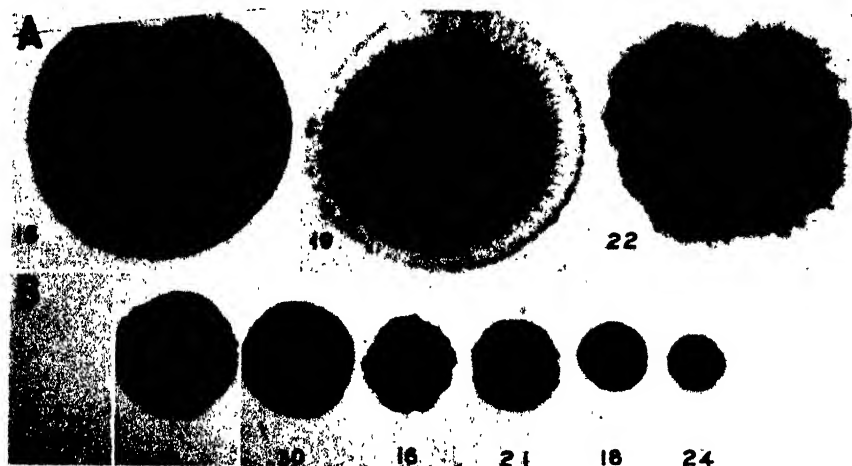


FIG. 1. Monoconidial cultures of *Venturia inaequalis* grown on malt-extract agar in Petri dishes at 21° C. The numbers indicate the different cultures. A. Grown for 48 days, note variations in margins. The colonies were grown in duplicate as indicated by the flattening at the top of the figure. B. Grown for 35 days.

Differences in colony margin were greatest when the cultures were grown under optimal conditions and they increased with the age of the colonies. On malt-extract agar the colony margins of most cultures were entire at temperatures above or below the optimum (16–22°). Marginal differences of 35-day-old colonies (Fig. 1, B) were not so great as those of the 48-day-old colonies shown in figure 1, A, where entire, filamentous, and lobate margins were very marked.

The several cultures grown under uniform conditions differed greatly in the size of the colonies produced in a given length of time. (Fig. 1, B.) Rate of growth is considered more fully under physiologic studies. In color the colonies of different cultures varied from a light mouse gray to a dark olive gray. The color is largely dependent on the amount of aerial mycelium, which varies with the medium and the culture. Wiesmann (17) has described similar differences in colony types of five isolations of *Venturia inaequalis*.

*Spore Production.* Certain cultures of *Venturia inaequalis* produced conidia more abundantly than others under uniform conditions. Records were made of the production of conidia and the initiation of perithecia in stock cultures of certain cultures grown on malt-extract agar in test tubes kept in an ice-box at 8°. The stock cultures were in duplicate and in each case both tubes conformed closely in the number of spores and perithecia when small portions of the agar and mycelium were examined under the microscope. No mature perithecia have been found in these cultures. In one series cultures were observed after 2 months and in others after 9 and 12 months. All degrees of spore production were found. The cultures were quite constant in spore production under the conditions of the experiment. As might be expected, the older cultures in certain cases showed fewer conidia and more perithecia. Examinations of cultures grown on malt-extract agar during two temperature series have shown more abundant production of conidia at the temperatures from 8 to 16° than at 20° or above. Certain cultures produced some spores even at the higher temperatures, while others were almost free of spores at temperatures above 20°.

#### Physiologic Differences

Studies of physiologic differences between cultures of *Venturia inaequalis* included: (a) effect of source of isolation, temperature, hydrogen-ion concentration, and nitrogen source on the rate of growth; (b) differences in tolerance to toxic materials; and (c) stability in culture.

#### Differences in Rate of Growth

Differences in growth rate were easily noticeable when several cultures were grown in Petri dishes under standard conditions. Duplicate series, after 35 days on malt-extract agar at 21°, gave consistent results and revealed that the average diameter of the fastest growing culture was 40 per cent larger than that of the slowest growing culture.

*Relation of Source of Isolation.* With the object of finding whether any relation existed between the rate of growth and the apple variety from which the cultures were isolated or the locality from which they came, an experiment was set up using 13 cultures isolated from 4 varieties of apple secured from 4 States. The liquid medium used for the experiment consisted of the following ingredients in the stated concentrations: M/4 dextrose, M/5 KNO<sub>3</sub>, M/20 KH<sub>2</sub>PO<sub>4</sub>, M/100 MgSO<sub>4</sub>, and a trace of FePO<sub>4</sub>. This solution in 10 cc. amounts was placed in 50 cc. flasks and steamed 1 hour on each of 2 successive days. Four flasks were planted with small equal portions of mycelium from each of the 13 cultures and were then kept at a constant temperature of 21° for 45 days. The mycelium was transferred to filter papers on a Büchner funnel and washed with distilled

water. After drying at 65° for 22 hours each filter paper, with the contents, was weighed.

In table 1 the cultures are arranged according to their rate of growth. Culture 30 from New York made the best growth while culture 27 from

TABLE 1.—*Relation of source of monoconidial cultures to rate of growth, production of conidia and perithecia in culture, and pathogenicity*

Culture number	Source of culture	Dry weight of mycelium <sup>a</sup>	Abundance of conidia <sup>b</sup>	Abundance of perithecia <sup>b</sup>	Reaction on Y. Transparent <sup>c</sup>
		<i>Mgm.</i>			
30	Fameuse N. Y.	93	+++	+	No infection
26	McIntosh Mich.	87	+	++	Infection
35	Northern Spy Ore.	68	+	+	.....
33	Yellow Newtown Ore.	67	++	+++	No infection
31	Northern Spy Ore.	65	+++	+	.....
21	McIntosh Wis.	58	+	+	Infection
18	Yellow Newtown Ore.	56	++	++	No infection
20	Fameuse Wis.	53	+	+	.....
17	Fameuse Wis.	49	+++	+	No infection
36	Fameuse Wis.	42	+++	++	.....
19	Yellow Newtown Ore.	38	+++	+	Infection
24	Northern Spy Mich.	36	+	+	Infection
27	McIntosh N. Y.	24	+++	+	.....

<sup>a</sup> Dry weight of mycelium in mgm. after 45 days in synthetic solution at 21°.

<sup>b</sup> Relative abundance of conidia and perithecia present after 2 months on malt-extract agar at 8°; +++ = abundant; ++ = moderate; + = sparse.

<sup>c</sup> Results of inoculations on Yellow Transparent trees in the greenhouse.

New York made the least. The 3 cultures isolated from Yellow Newtown apples from Oregon showed much variation. Culture 33 made a growth almost twice that of 19. The abundance of conidia and perithecia and ability to infect leaves of Yellow Transparent have been added to the table as they give further evidence that variations in cultures isolated from a single variety and locality are as great as between cultures from widely separated localities or different varieties. Cultures 18 and 19 were isolated at the same time from the same collection of apples, yet their characteristics are quite different. For instance, of the three isolations from Yellow New-

town apples from Oregon, cultures 33 and 18 failed to infect Yellow Transparent leaves, while culture 19 succeeded. Culture 33 initiated abundant perithecia; 18, a moderate number; and 19, only a few. While the number of cultures studied in this experiment was not large, the results indicate wide variation between cultures from a given locality and variety.

Wiesmann (17) found variations in growth rate, color, and shape of the mycelium when pure cultures of *Venturia inaequalis*, isolated from different apple varieties in the same locality, were grown in dilute pear and grape juice media. From pure culture studies he concluded that isolations made from conidia taken from infected twigs of a given host did not differ from isolations of conidia from leaves of the same host. His results agree with the findings of the present writer in indicating the presence of several widely different strains of *V. inaequalis* in one locality.

*Effect of Temperature.* Keitt and Jones (10) give 16° to 22° as the optimal temperature for ascospore germination and germ-tube growth. This temperature range also was found favorable for the growth of the mycelium on agar media and for leaf infection of the host plants. Studies made by the present writer to determine whether cultures had different temperature requirements revealed that the isolations considered had the same optimal temperature for growth but at this temperature the cultures varied greatly in their rates of growth.

Eight cultures were used for a study of the effects of temperature on growth rate. For the first series malt-extract agar was used with the addition of 2.4 grams of  $MgSO_4$  and 6.8 grams of  $KH_2PO_4$  per liter to help buffer the medium. Petri dishes containing 25 cc. of this medium were inoculated at their centers with small portions of mycelium. The dishes were then bound with adhesive tape to prevent drying at the higher temperatures. Five plates of each of six cultures (1, 16, 18, 21, 24, and 30) were placed at the following temperatures for 5 weeks: 7, 17, 21, and 27°. variable 1°. At the temperatures considered, culture 24 consistently grew more slowly than any other; no culture, however, grew consistently faster than the rest. At 21° culture 1 produced the largest colonies, at 27° colonies of culture 30 were the largest, and at 7° cultures 16 and 21 grew faster than the others. Each culture grew most rapidly at 21°, which is near the optimal temperature for mycelial growth of *Venturia inaequalis*. The average colony diameters at 21° were: Culture No. 1, 50 mm.; No. 16, 37 mm.; No. 18, 26 mm.; No. 21, 35 mm.; No. 24, 26 mm.; and No. 30, 45 mm.

A second series differed from the first by the omission of  $MgSO_4$  and  $KH_2PO_4$  from the medium and by the substitution of wide rubber bands for the tape to prevent drying. This series included cultures 19, 22, 24, and 30 at each of the following temperatures: 4, 8, 12, 16, 20, 24, 28, and

32°, with a variability of 1°. The replications gave consistent results and are averaged in table 2, which shows the final diameters of the colonies of each culture at the different temperatures at the end of 5 weeks.

TABLE 2.—*Effect of temperature on the diameter of colonies of four monoconidial cultures of Venturia inaequalis on malt-extract agar*

Temp. ° C.	Diameter of colony in mm. after 5 weeks for stated cultures			
	19	22	24	30
4	6	8	4	14
8	12	10	9	21
12	17	18	12	30
16	17	18	14	34
20	20	22	15	44
24	19	16	12	35
28	10	1	1	11
32	0	0	0	0

In the second series all four cultures grew throughout a temperature range from 4° to 28°, but none grew at 32°. Culture 30 produced the largest and culture 24, the smallest colonies at each temperature. Cultures 22 and 19 showed similar growth rates at the lower temperatures but 22 grew slower at the higher temperatures than 19. Although the optimal temperature for growth on malt agar for all the cultures studied lies near 21°, yet, at this temperature, certain cultures grew twice as rapidly as others. The relative rates of growth of the cultures change at temperatures above and below the optimum.

*Effect of Hydrogen-ion Concentration.* Three series of experiments have been made to find whether the cultures have different H-ion relations. In the first series two cultures were used, 18 that had given infection on Wealthy and Fameuse, but none on Yellow Transparent leaves, and 21, which had given good infection on all 3 varieties. The cultures were grown in a modified Czapek's solution to which a mixed buffer solution was added, the pH being varied by the addition of increasing amounts of sodium hydroxide as described by MacInnes (12). Flasks containing 56 cc. of the medium were sterilized and planted with small equal portions of mycelium. Culture 21 was duplicated in a series exactly the same as the first, except that the flasks were planted with spore suspensions. After 47 days, at 21°, the mycelium was removed from solution to weighed filter papers in a Büchner funnel, washed with distilled water, and dried for 26 hours at 65°. (Table 3).

Another pH series was set up using malt-extract solution inoculated with 5 different cultures. Cultures 24 and 21 gave infection on Yellow Transparent leaves in 1930, while the other 3, cultures 16, 18, and 30, gave no infection on Yellow Transparent leaves but did infect those of Wealthy and Fameuse. The nutrient solution was made up in one lot as follows:

Trommer's malt extract .....	125 grams
$\text{KH}_2\text{PO}_4$ .....	34 grams
$\text{MgSO}_4$ .....	12 grams
$\text{H}_2\text{O}$ .....	5000 cc.

This solution was divided into 5 lots and normal HCl or NaOH was added to bring them to the proper pH values. The solution in 50 cc. amounts was then placed in 250-cc. flasks and sterilized at 12 pounds' pressure for 30 minutes. Five flasks were planted with each culture for

TABLE 3.—*The effect of hydrogen-ion concentration on the growth of certain monoclonal cultures of Venturia inaequalis*

Culture Series 1	Average dry weight of mycelium in mgm. in Czapek's solution of varying pH after 47 days at 21°					
	2.6	3.6	5.0	6.6	8.6	9.4
No. 18	1.8	35.3	61.6	12.6	8.0	5.6
No. 21	3.0	17.0	22.3	10.6	9.5	8.3
No. 21 <sup>a</sup>	2.5	21.5	33.6	9.0	0.0	0.0
Series 2	Average dry weight of mycelium in mgm. in malt extract of varying pH after 41 days at 21°					
	3.3	3.8	4.8	5.4	6.0	
No. 16	103	284		291	212	
No. 18	80	168	182	209	145	
No. 21	47	97	113	233	135	
No. 24	70	72		105	92	
No. 30	100	167		158	147	
Series 3	Average diameter increase in mm. of colonies on malt-extract agar of varying pH after 35 days at 20°					
	3.4	4.8	5.4	6.4	7.4	
No. 15	12	17	18	17	15	
No. 18	24	24	27	26	23	
No. 19	13	20	21	16	17	
No. 22	14	21	23	19	16	
No. 24	16	22	22	17	14	
No. 30	43	37	35	31	24	
No. 33	29	41	39	31	25	

<sup>a</sup> Started with spores instead of mycelium.

each pH. Mycelium on malt-agar slants was cut up and introduced with about 2 cc. of water into the flasks, which were kept at 21° for 41 days. Results were taken by weighing the mycelium as in the previous series, (Table 3).

A third hydrogen-ion series was set up, using a buffered malt-extract agar as the medium, so that the growth rate might be followed throughout the experiment. The buffer materials were added to the agar in the following amounts:

Citric acid	8.40 grams	Agar	80.00 grams
Glycine	3.04 grams	H <sub>2</sub> O	3500.00 cc.
KH <sub>2</sub> PO <sub>4</sub>	3.63 grams		

These materials were dissolved and then divided into 10 lots of 340 cc. and brought to the required pH by adding approximately normal NaOH in suitable amounts, so that the range extended from pH 3.4 to 7.4. The malt extract (100 grams in 250 cc. of water) was sterilized separately and added to the buffer-agar mixture after sterilization in 25 cc. amounts with enough sterile water to bring the total volume of each flask to 400 cc. Sixteen Petri dishes were then poured from each flask so that every dish contained about 25 cc. of the medium. Samples from each flask were saved and the pH determined by a quinhydrone electrode.

The seven cultures used in this study were placed on the medium as 4 mm. discs cut from 10-day-old plate cultures with a stiff wire loop. Two discs of the same culture were placed in each Petri dish. Cultures 18, 19, and 24 were repeated 6 times at each pH, while 15, 22, and 33 were repeated 4 times. Culture 30 had only 2 colonies at each pH, except at pH 3.4 and pH 5.4, where 4 colonies were grown. The diameters of the colonies were measured every 7th day for a period of 5 weeks. The results are shown in table 3, which gives the final colony diameters of the seven cultures at each hydrogen-ion concentration.

The medium was tested at the end of the experiment for any change in pH. A slight increase in pH was found throughout the entire range, except the medium with an initial pH of 7.4, which had changed very little. The medium on which culture 18 had grown showed a greater change in pH throughout the series than the corresponding medium on which the slower growing culture 15 had developed. Also it was observed that the greatest change in hydrogen-ion concentration was found at the most favorable pH for growth of the cultures, which indicates a direct correlation between the amount of fungus growth and the change in pH.

*Venturia inaequalis* grew in a modified Czapek's solution throughout a range from pH 2.6 to 9.4. Conidia of culture 21 germinated in solutions ranging from pH 2.6 to 6.6 but did not germinate in solutions of pH 8.6 or

9.4. When grown in malt-extract solution at 20° cultures 16, 21, 18, and 24 grew better at pH 5.4 than at pH 4.8 or 6.0. Culture 30 grew slightly better at pH 3.8 than at pH 3.3 or 5.4. When grown on malt-extract agar at 20° cultures 19, 22, and 24 grew best at pH 5.4, while cultures 18 and 15 made slightly better growth at pH 5.6. Culture 33 grew best at pH 4.8 and 30 at pH 3.4. Certain cultures have been included in each of the series, and the results obtained in the 2 series check favorably.

The optimal hydrogen-ion concentration for all the cultures studied lies between pH 4.8 and 5.6 with the exception of culture 30, which grew better at pH 3.4 than at the higher hydrogen-ion concentrations. Even though certain cultures have the same optimal hydrogen-ion concentration for growth, there is much difference in the rate of growth of the cultures at that concentration.

Wiesmann (17) found that "races" of *Venturia inaequalis* behaved differently on yeast extract at the neutral point and with various amounts of tartaric acid added. His Virginia "form" grew best on the neutral medium, while Grossherzog, Wellington, and Boiken forms grew best on solutions made acid with 0.1, 0.2, and 0.3 per cent concentrations of tartaric acid, respectively.

*Effect of Nitrogen Source.* The source of nitrogen has a marked influence on the growth of *Venturia inaequalis*. In order to determine whether cultures varied in their response to nitrogen source, they were grown on nutrient solutions containing nitrogen supplied by ammonium sulphate, potassium nitrate, and calcium nitrate, respectively. Two series were run in which the following materials were used in amounts to give the indicated dilutions: Dextrose M/3,  $\text{KNO}_3$  M/5  $\text{KH}_2\text{PO}_4$  M/20,  $\text{MgSO}_4$  M/100,  $\text{FePO}_4$  trace. This solution was used in 50 cc. portions in 250 cc. flasks with  $\text{Ca}(\text{NO}_3)_2$  M/5 or  $(\text{NH}_4)_2\text{SO}_4$  M/5 substituted for  $\text{KNO}_3$ , as indicated in table 4. The flasks were planted with small portions of mycelium in the first series, and with spore suspensions in the second series, then kept at 20° for 45 days. The results are given in table 4 as the oven dry weight of the mycelium (dried 24 hours at 80°).

Cultures 18 and 21 both grew best when  $(\text{NH}_4)_2\text{SO}_4$  was used as the source of nitrogen and both made about the same amount of growth on this solution. Culture 18 made better growth in the  $\text{Ca}(\text{NO}_3)_2$  than in the  $\text{KNO}_3$  solution, while culture 21 grew much better in the  $\text{KNO}_3$  solution. Cultures 17, 19, 24, and 30 reacted like 21 on these solutions. No other culture has been found that reacts like 18 in preferring  $\text{Ca}(\text{NO}_3)_2$  to  $\text{KNO}_3$ .

#### DIFFERENCES IN TOLERANCE TO TOXIC MATERIALS

Certain chemicals used in fungicides are toxic to *Venturia inaequalis* and when placed in malt-agar media in sufficient amounts will inhibit



growth of the fungus. The following experiments were made in order to determine whether the percentage of the toxic material necessary to inhibit growth would vary with the different strains of the fungus.<sup>4</sup>

The methods suggested by Schmitz (15) for toximetric studies of wood preservatives have been followed in these experiments and will be given only a brief description. Malt-extract agar was used as the nutrient medium and the chemicals were sterilized in part of the water and mixed with the agar just before being poured into the Petri dishes. Approximately 25 cc. of nutrient-chemical mixture was placed in each dish and planted with two 4-mm. discs of mycelium from 10-day-old cultures on malt-extract agar. The test plates were incubated for 3 weeks at 20°. Where no growth occurred on the plates or discs after 3 weeks the discs were transferred to standard malt-agar slants at 20° to determine whether the fungus was dead or alive. Then, if no growth occurred in 3 weeks, the fungus was considered dead.

Only materials employed as commercial sprays were tested. Sulphur dust and liquid lime-sulphur were tried in preliminary tests but were not satisfactory. Paris green and especially copper sulphate were much better adapted to this type of study and the results obtained with these materials will be presented.

Certain cultures were found to be much more tolerant to copper sulphate than others (table 5). Less difference in relative tolerance was found when Paris green was employed as a toxic agent.

#### STABILITY IN CULTURE

During the 3 years that the writer has had monoconidial cultures of *Venturia inaequalis* under observation, several have shown sudden changes in color, rate of growth, and spore production. Usually these changed characters were retained through asexual propagation. In other cases the cultures continued to change but seldom reverted to the original type. Other cultures were apparently more stable as no changes were observed to occur in them. The variations usually appeared as fan-shape sectors starting at or near the center of the colony.

As far as the writer has observed, sectoring in *Venturia inaequalis* is usually associated with loss of spore production by the sectors. The sectors usually grow more rapidly than the original colony and differ slightly in color. By placing the cultures at temperatures of 12 to 16° the sectors usually produced a few spores, which sometimes appeared abnormal because of odd shapes and the habit of germinating before being cut off. Culture 19 is shown in figure 2 with a sterile sector. The original colony was sporu-

<sup>4</sup> With the permission of Dr. G. W. Keitt, these data are taken from studies of the toxicity of various substances to *V. inaequalis*, in which the writer assisted.

lating freely (Fig. 2, C), while the sector was producing only a very few conidia (Fig 2, D) after 20 days at 12°.

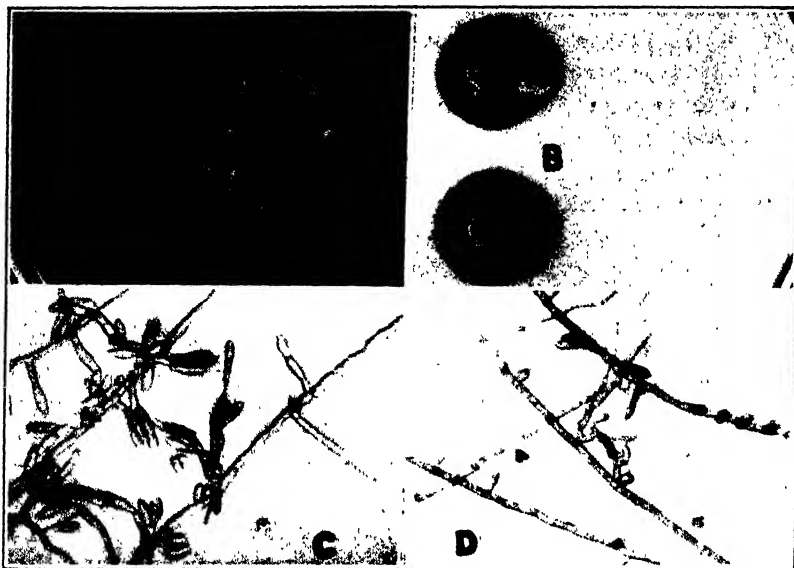


FIG. 2. Sterile sectors of culture 19 grown on malt-extract agar in Petri dishes. A. Three weeks at 20° C. followed by 20 days at 12° C. B. Found in pH series at pH 3.4 after 35 days at 20° C. C. Sporulating margin of the lower part of colony shown in A. D. Sterile margin of sector shown in A. C. and D.  $\times 160$ .

When portions of these sterile sectors were transferred to Petri dishes and compared with the original culture the colonies were usually larger, more dense, and of slightly different color. Sometimes these sterile cultures produced other sectors differing in color and rate of growth. None have been observed to regain their ability to produce conidia abundantly.

TABLE 4.—Effect of nitrogen source on monoconidial cultures of *Venturia inequalis* in nutrient<sup>a</sup> solution for 45 days at 20° C.

Nitrogen source	Series	Culture 18		Culture 21	
		Flasks used	Average weight of mycelium	Flasks used	Average weight of mycelium
		No.	Mgm.	No.	Mgm.
KNO <sub>3</sub> .....	First	3	31	3	42
“ .....	Second	15	31	15	45
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	First	2	59	5	18
“ .....	Second	5	44	5	11
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	Second	5	100	5	102

<sup>a</sup> The composition of the medium used is given in the text.

Aderhold (1) observed that the mycelium of *Venturia inaequalis*, in culture, undergoes a gradual differentiation into two types. In one type he found sparse mycelial growth and abundant sporulation; in the other, an abundant mycelium was produced and only a few spores. He considered that lack of nutrient was a likely cause. He also reported that towards autumn the richness of spores diminished. In winter he found little conidial production but much mycelium. However, he grew his cultures at 25°, while the writer found production of conidia most abundant at temperatures below 20°. Stock cultures on malt-extract agar kept at 8° in an ice-box were still sporulating freely after 3 years. As shown earlier in this paper, certain strains always produce spores more abundantly than others.

Besides the sectoring colonies mentioned, other indications of instability in culture have been noted. Culture 16, used in the inoculation series of 1930, later produced so few conidia that its use in this work had to be discontinued. Culture 21 gave good infection during 1930 on 3 varieties but, in 1931, no infection was obtained with conidia of this culture on these or

TABLE 5.—Percentage of copper sulphate or Paris green in the culture medium tolerated by different monoconidial cultures of *Venturia inaequalis*

Culture used	Replications averaged	Per cent of CuSO <sub>4</sub> in medium:					
		0.06	0.08	0.10	0.12	0.14	0.16
	No.						
17	4	++ <sup>a</sup>	+	—	—	—	—
30	4	++	++	+	+	+	—
22	12	++	++	+	+	—	—
15	6	++	+	—	—	—	—
18	6	++	++	++	++	+	—
24	8	+	+	—	—	—	—
33	4	++	++	+	—	—	—
		Per cent of Paris green in medium:					
		0.10	0.18	0.22	0.26	0.30	0.34
17	4	++	+	+	+	—	—
30	4	++	++	++	++	++	++
22	12	++	++	+	+	+	+
15	6	++	++	+	+	+	—
18	6	++	+	+	+	—	—
24	8	++	+	+	+	+	—
33	4	++	++	++	+	+	+

<sup>a</sup>++ = measurable growth on the toxic medium in three weeks; + = no measurable growth on the toxic medium but growth when transferred to malt-agar slants after three weeks on the toxic medium; — = fungus killed, no growth when transferred to malt-agar slants after three weeks on the toxic medium.

other varieties. This culture, apparently, was no longer pathogenic. A possible explanation of such changes would be the chance transfer of portions of sterile or nonpathogenic sectors arising in stock cultures where their presence would not be readily noticed.

Since certain of the cultures appeared to be changing, reisolations were made of several of the cultures to ascertain whether single conidia of the same culture would produce similar colonies or colonies differing in certain characters. Eighteen single conidia of culture 22 and 6 single conidia of culture 24 were isolated in the manner described earlier and allowed to develop on malt-extract-agar slants. After 10 days transfers were made to Petri dishes containing malt-extract agar. Two small portions of mycelium developed from single conidia were then placed in the Petri dishes. These cultures were kept at 20° for three weeks and then compared in general appearance, size of colony, and color. All single-spore colonies from each culture were consistent in all the above characters.

#### STUDIES OF PATHOGENICITY

Conidial inoculations were made on 20 commercial apple varieties and 6 closely related genera to determine the different infection capabilities of certain monoconidial cultures of *Venturia inaequalis*.

#### METHODS AND MATERIALS

*Spore Production.* The spores used for inoculations were produced on cheesecloth wicks in a nutrient solution. Cheesecloth was cut into pieces about 5 by 10 cm. and placed in test tubes so that it formed a lining for the tubes. About 3 cc. of nutrient solution (25 grams of Trommer's malt extract in one liter of water) was introduced into each tube. The tubes were then sterilized in the autoclave at 15 pounds' pressure for 20 minutes. About 2 weeks before the spores were needed, the tubes were inoculated by placing bits of mycelium from malt-agar slants on the cheesecloth wicks and spreading it over the surface of the wicks with a sterile needle. Care was taken not to introduce any more agar than necessary. After inoculation the tubes were kept at 20° for a week and then placed at 14 to 16° until needed, as the lower temperature favored spore production.

When a spore suspension was needed for inoculations the cheesecloth wicks were removed from the tubes and placed in the top part of a Petri dish fixed in an upright position and standing inside the lower half of the dish. Distilled water was used in an atomizer to wash the spores from the wicks into the dish. The abundance of spores varied with different strains, so more water was added to the heavily sporulating cultures until the spore suspensions of all were of about the same concentration. All suspensions were adjusted to an average of 10 spores per low-power field of the microscope. The trees were inoculated by spraying them with the spore suspen-

sion by means of an atomizer. Both the upper and lower surfaces of the leaves were covered as evenly as feasible.

*Care of the Trees.* One- and 2-year-old potted trees limited to 2 or 3 twigs each were used for these studies. All the trees were, so far as possible, kept in the same greenhouse and under the same environmental conditions. Trees with the same amount of shoot growth were used for a given series. After inoculation, the trees were placed in the moist chamber, devised by Keitt (10), at a temperature of 16° to 20° where they remained for 48 hours, unless otherwise stated.

When removed from the moist chamber the trees were placed in the greenhouse. After 3 weeks had been allowed for the disease to develop, records were made of the scab lesions on each infected leaf. The maximum number of lesions recorded on any one leaf was 100, regardless of any greater amount of infection, as counts could not be made with satisfactory accuracy if more than 100 lesions were present, and a quantitative record above 100 would add little value to the data.

#### INFECTION STUDIES ON CERTAIN SPECIES RELATED TO APPLE

Aderhold (2) reported *Fusicladium dendriticum* on the following *Pyrus* species: *P. spectabilis* Ait., *P. kaido* Sieb., *P. floribunda* Sieb., *P. baccata* L., *P. prunifolia* Willd., *P. rivularis* Hook., and *P. dioica* Mnh. In 1903 Aderhold (4) recorded cultural experiments that showed *Fusicladium crataegi* Adh. to be distinctly different from *F. dendriticum*. Aderhold concluded that the fungus on *Crataegus* was entirely distinct from that on apple and that cross infections were unlikely. He also described experiments by which he determined that *F. orbiculatum* Denn. on *Sorbus torminalis*, which is morphologically closely related to *F. dendriticum*, cannot infect apple.

Very little recent work has been reported on this phase of the apple-scab problem. In order to extend the knowledge on this phase and to determine the infection capabilities of different cultures on the several hosts a series of experiments was started in which 29 species representing 6 genera closely related to the apple were inoculated with 6 monoconidial cultures of *Venturia inaequalis*.

*Results of Inoculations on 6 Genera.* During the spring of 1931 the following genera were inoculated as potted trees in the greenhouse; 13 species of *Malus*, 9 of *Crataegus*, 3 of *Sorbus*, 2 of *Amelanchier*, 1 of *Aronia*, and 1 of *Cotoneaster*. These plants were inoculated with cultures 15, 17, 18, 19, 24, and 30. Two or more trees of each species were used in each series. All these plants were obtained from the Kelsey-Highlands Nursery, East Boxford, Massachusetts. Only 9 of the 13 *Malus* species inoculated were infected by the cultures studied, the other 4 remained entirely resistant to all

6 of the cultures. None of the 5 genera, other than *Malus*, showed any signs of infection. The results of this work are in accord with those of Aderhold in that infection by *Venturia inaequalis* occurred only on hosts within the genus *Malus*.

*Results of Inoculations on Malus Species.* The *Malus* species studied showed some interesting reactions to the different cultures used for inoculations (Table 6). The 9 species infected by 1 or more of the cultures can

TABLE 6.—*Results of infection experiments with monoconidial cultures of Venturia inaequalis on certain Malus species*

Species of <i>Malus</i> inoculated	Culture number						Cultures used	Cultures infecting
	15	17	18	19	24	30		
<i>niedzwetzkyana</i> .	+ <sup>a</sup>	+	+	+	+	+	No. 6	No. 6
<i>ioensis</i> . . . . .	+	...	+	...	+	+	4	4
<i>robusta</i> . . . . .	..	...	+	...	+	+	3	3
<i>baccata</i> . . . . .	+	0	+	+	0	+	6	4
<i>sieboldii</i> . . . . .	+	+	0	0	0	0	6	2
<i>toringoides</i> . . . . .	+	+	..	+	.	0	4	3
<i>coronaria</i> . . . . .	...		+		0	+	3	2
<i>sargentii</i> . . . . .	.	...	+	0	0	0	4	1
<i>tschonoskii</i> . . . . .	+	..	0	0	0	0	5	1
<i>theifera</i> . . . . .	0	0	0	0	0	0	6	0
<i>angustifolia</i> . . . . .	0	0	0	0	0	0	6	0
<i>floribunda</i> . . . . .	0	0	0	0	0	0	6	0
<i>arnoldiana</i> . . . . .	0	0	0	0	0	..	5	0

<sup>a</sup> This experiment was conducted on potted trees in the greenhouse; + = infection; 0 = no infection.

be placed in 2 groups: those infected by all of the cultures, and those infected by some and resistant to others. Those species infected by all of the cultures include: *M. niedzwetzkyana* infected by all 6 cultures, *M. ioensis* infected by 4, and *M. robusta* infected by 3. *M. baccata*, *M. sieboldii*, *M. toringoides*, *M. coronaria*, *M. sargentii*, and *M. tschonoskii* were each infected by 1 or more of the cultures and resistant to others. The 4 species entirely resistant to all 6 cultures were: *M. theifera*, *M. angustifolia*, *M. floribunda*, and *M. arnoldiana*. A study of a larger number of cultures may reveal some that are capable of infecting these other *Malus* species.<sup>5</sup>

<sup>5</sup> The source and identity of the species here concerned as furnished by Harlan P. Kelsey, Jr., is as follows: *Malus niedzwetzkyana*, obtained from grafts from original stock from Arnold Arboretum; *M. ioensis*, grafts from A. A.; *M. robusta*, grafts and buds from A. A.; *M. theifera*, buds and grafts from A. A.; *M. arnoldiana*, buds and grafts from A. A.; *M. baccata*, buds from A. A.; *M. toringoides*, buds from A. A.;

Reisolations of the fungus were made from all the species infected, except *Malus tschonoskii*, and the cultural characters in each case appeared the same as in the original culture. When spores of these reisolated cultures were used to inoculate varieties of *Malus malus*, good infection was obtained.

*Plant Variations.* In working with these *Malus* species some variations in the plants were noted. The plants were not so uniform as the commercial apple varieties used for infection studies. Ten trees of each species were potted and were usually similar in morphological appearance but in each of 4 species certain trees showed different pathogenic reactions. One example will suffice to indicate the kind and amount of variation found. Ten trees of *Malus baccata* were inoculated with 6 different cultures and the results obtained are shown in table 7. For convenience the trees are given refer-

TABLE 7.—Variation in resistance to infection as shown by ten trees of *Malus baccata* inoculated with monoconidial cultures of *Venturia inaequalis*

Culture number	Individual trees									
	A	B	C	D	E	F	G	H	I	J
15 ..					0				+	
17 ....			0				0			
18 ..	+			0				0	0	+
19 ..	+									+
24 ..	0				0	0	0	0		0
30 ...		+		0						

ence letters from A to J. Some of the trees were infected by a given culture, while other trees were not infected by the same culture. Trees A and J were infected by cultures 18 and 19 but not by 24, while trees C, D, E, F, G, and H were not infected by any of the cultures. Tree I was infected by culture 15, while tree E was not nor was I infected by culture 18, which infected trees A and J. Culture 30 was able to infect tree B but did not infect tree D. *Malus robusta*, *M. sargentii*, and *M. coronaria* showed similar variations in susceptibility. It is apparent that in these cases both the susceptible and the pathogen are quite variable. Such variations as are shown in table 7 between cultures 18 and 24 or 15 cannot be disregarded. These differences in the infection capabilities of cultures on certain *Malus* species, when considered with similar differences on the less variable apple varieties, have been interpreted as showing the variability between certain cultures of *Venturia inaequalis*.

*M. sargentii*, seeds from A. A.; *M. angustifolia*, grafts from A. A.; *M. sieboldii*, seeds from A. A.; *M. coronaria*, grafts from Chase Nursery Company, Chase, Alabama; *M. tschonoskii*, seeds from Japan; and *M. floribunda*, grafts from Chase Nursery Company.

## INFECTION STUDIES ON CERTAIN APPLE VARIETIES

*Experiments of 1930.* During the spring of 1930 Yellow Transparent, Wealthy, and Fameuse trees were inoculated with conidia of 7 different cultures in 3 series of experiments. Yellow Transparent trees were not infected by 3 of the cultures but were heavily infected by the other 4. The Wealthy and Fameuse trees were infected by all of the cultures tried.

The data obtained are given (Table 8) for representative series. The 3

TABLE 8.—Results of infection experiments with monoconidial cultures of *Venturia inaequalis* on three apple varieties in 1930

Inoculation date and culture	Maximum number of lesions per leaf <sup>a</sup>		
	Yellow Transparent	Fameuse	Wealthy
April 1			
Wis. 15 . . . . .	0	31-68	5-19
Ore. 19 . . . . .	100	100	15-96
Wis. 21 . . . . .	100	30-100	28-100
Wis. 22 . . . . .	9-100	1-14	7-29
April 11			
Ore. 18 . . . . .	0	18-26	
Ore. 19 . . . . .	100	100	100
Wis. 21 . . . . .	50-100	20-27	31-100
Wis. 22 . . . . .	25-100	46-60	27-100
Mich. 24 . . . . .	100	100	100
April 25			
Wis. 16 . . . . .	0	100	100
Ore. 18 . . . . .	0	52-83	13-70
Wis. 21 . . . . .	23-36	18-43	19-36
Wis. 22 . . . . .	28-49	38-46	20-42
Mich. 24 . . . . .	100	40-64	53-100

<sup>a</sup> Four shoots on two or more trees were inoculated with each strain.

cultures (15, 16, and 18), which did not infect Yellow Transparent, were consistent in never producing lesions on leaves of this variety. Cultures 19, 21, and 24 produced abundant infection on all 3 varieties but usually showed more lesions on the Yellow Transparent than on the Fameuse or Wealthy trees. Culture 22 also infected all 3 varieties but not so heavily as the other 3 cultures.

*Experiments of 1931.* In the spring of 1931 further studies of pathogenicity were made, which included 20 varieties of apples inoculated with 9 cultures of *Venturia inaequalis*. These studies were made in a manner similar to that of 1930, the main difference being the use of only 1 culture



in a series instead of 4 or 5, as used during the previous season. This change made it possible to treat a large number of varieties in a series without danger of mixing the inoculum while the trees were in the inoculation chambers. Most series were repeated at least once and in all cases the results were quite consistent. Where no infection is reported macroscopic scab lesions were not present on any of the leaves. When vigorous trees were used either all the shoots showed infection or none of them were infected, depending on the variety and culture used.

TABLE 9.—*Results of infection experiments with monoconidial cultures of Venturia inaequalis on certain apple varieties in 1931*

Variety inoculated	Culture tested								
	Wisconsin			Oregon			Michigan		N. Y.
	15	17	22	18	19	33	24	26	30
Yellow Transparent	0	0	+	0	+	0	+	0	0
McIntosh	0			0	+	0	+		0
Dudley	+			0	0		+		+
Hubbardston				+	0	0	+		+
Missouri Pippin	0	0	+	+	0	+	+		+
Fameuse	+	+	+	+	+		+	+	+
Wealthy	+	+	+	+	+		+		+
York Imperial				+					+
Haralson		+		+	+	+			+
Baldwin			+	+	+				+
Red Astrachan		+		+		+			+
Early Harvest		+		+		+			+
Ben Davis		+		+		+			+
Ingram		+		+		+			+
Pewaukee				+	+		+		+
Golden Delicious					+				+
Twenty Ounce				+	+				+
Price Sweet		+				+			+
August Strawberry King				+		+	+		+

The results of the inoculation experiments on apple varieties are shown in table 9, which represents a total of over 600 twigs including duplicate series, inoculated with 9 monoconidial cultures during 1931. Since this study included 20 varieties inoculated with 9 different cultures in several series, the problem of presenting the detailed data was greatly increased. Therefore, only a summary is given.

The varieties studied may be divided into 2 groups according to their susceptibility. The first group is composed of those that were susceptible in some degree to all of the cultures with which they were inoculated. The

second group is composed of those varieties infected by some cultures but not by others. Of the 20 varieties studied, 15 were more or less susceptible to all the cultures with which they were inoculated. However, there was a wide difference in the degree of infection among these varieties. Certain varieties like Fameuse, Red Astrachan, and Early Harvest showed considerable infection with each of the cultures used. Other varieties, as, for example, Ingram and Haralson, were abundantly infected by some cultures and only sparsely infected by others.

The second group was composed of 5 varieties that were not infected by some of the cultures but were by others. Trees were designated free from infection when no macroscopic scab lesions were apparent 3 weeks after inoculation, when the records were taken, or thereafter as long as the trees were under observation. Culture 30 when used on Yellow Transparent trees sometimes caused flecks to appear on the leaves but these disappeared after a few days. Modified treatments in the inoculation chambers were tried in attempts to produce normal lesions and spores but without success. Therefore this variety has been considered free from infection by culture 30 under the conditions of the experiments.

Yellow Transparent, McIntosh, Dudley, Missouri Pippin, and Hubbardston Nonsuch were the varieties not infected by certain cultures. Table 9 shows the cultures that were able to infect each of these varieties. From these data it can be seen that some varieties are susceptible to certain cultures of the fungus from a given region but resistant to other cultures from the same region.

Table 9 shows clearly that the cultures differed in their infection capabilities on certain varieties. Culture 24 from Michigan was able to infect all varieties on which it was used and differed in this respect from all the other cultures studied. Culture N. Y. 30 differed from Ore. 18 by infecting Dudley. Ore. 19 differed from both N. Y. 30 and Ore. 18 because it was able to infect Yellow Transparent and McIntosh but not Missouri Pippin and Hubbardston Nonsuch, which were infected by 30 and 18. Wis. 17 differed from culture 18, 30, and 33 because it did not infect Missouri Pippin. Culture 15 from Wisconsin differs from 18 and 19 from Oregon by causing abundant infection on Dudley. The 1931 results confirmed those obtained in 1930 and further indicated strain specialization of *Venturia inaequalis*.

Wiesmann (17) has reported specialization in *Venturia inaequalis*. Although he obtained mostly negative results when leaves were inoculated with conidia from pure cultures, when conidia from a given host were used they were reported to be 2 to 4 times as virulent on leaves of the same variety as on those of other varieties. Most of his infection studies were made with cut leaves of 4 varieties: Wellington, Boiken, Virginia, and Gravenstein; and each variety was partly susceptible to infection by conidia from

the other 3 varieties. His report that none of these varieties was immune from conidia of any race might be explained by the fact that only 4 were studied and that the conidia were obtained from leaves and not from pure monoconidial culture and, therefore, might have contained more than one strain. In his infection studies the writer found that most of the varieties used were partly susceptible to all cultures, but 5 varieties were not infected by certain cultures, though susceptible to others.

#### STUDIES OF MORPHOLOGY

Besides the differences found in the cultural and infection studies certain cultures also showed slight morphologic differences. For this study the length of conidia was used as a criterion of culture differences. The conidia of *Venturia inaequalis* are rather variable in size, even when obtained from infected leaves. Aderhold (1) gave the length of conidia as 16 to 25 microns and the width as 6 to 10 microns. Most spores had a length of 18 to 22 microns and width of 8 to 10 microns. Wiesmann (17) compared the length of conidia taken from leaves of 4 apple varieties. By measuring 500 spores, each, from leaves of Wellington, Virginia, Gravenstein, and Boiken he found that the peak of curves drawn from the spore lengths fell at 24, 18.6, 20, and 21.2 microns, respectively. These results he concluded gave further proof that the scab strain on each of the above apple varieties was a "specialized race."

*Conidial Length in Culture.* In one part of the writer's study the spores were obtained from pure cultures grown under uniform conditions. The spores were produced on cheesecloth wicks in malt-extract solution, as described in the methods used during the study of pathogenicity. The conidia were mounted in glycerine and 300 spores from each of the 7 cultures were measured. By placing a stage micrometer under the high-power field of the microscope with a camera lucida attached, a scale divided into microns was drawn on white paper. Then, by replacing the stage micrometer with a slide of mounted spores, the length of the conidia could be measured directly in microns. A mechanical stage was employed so that no spores were measured twice.

The results of the measurements are shown in figure 3, A. It is easily seen that the typical spore length of 5 of the cultures falls in the range from 20 to 22 microns. Culture 19 produced smaller spores and its curve reached a peak at 18 microns. Culture 30 produced larger spores than any of the other cultures and its representative curve reached a peak at 27 microns, although many spores were much larger. Sixteen per cent of the spores of culture 30 and 11 per cent of the spores from cultures 15 were bicellular. The cultures also varied in spore shape. For example, cultures 18 and 19 produced spores with rounded ends in contrast to the pointed spores of the other cultures.

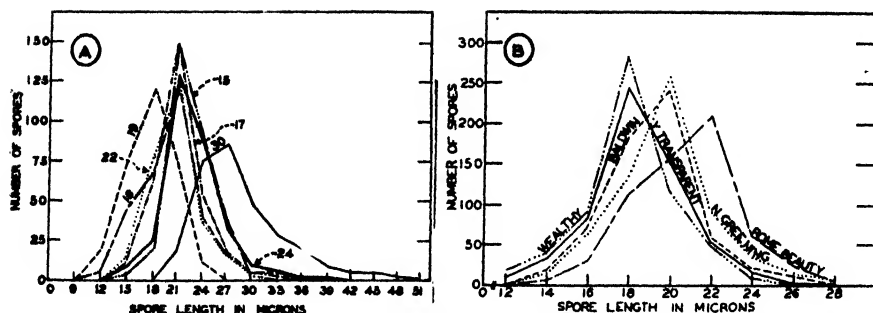


FIG. 3. A. The conidial length of 7 monoconidial cultures of *Venturia inaequalis* grown on malt-extract solution. Three hundred spores of each culture were measured under standardized conditions. B. The length of conidia of monoconidial culture 22 produced on Wealthy, Baldwin, Yellow Transparent, Northwestern Greening, and Rome Beauty trees in the greenhouse. Six hundred spores from each variety were measured under standardized conditions.

*Conidial Length on Certain Apple Varieties.* Preliminary study was made of spores washed from leaves of 3 varieties inoculated with culture 19 in the greenhouse during the infection studies of 1931. The interesting fact suggested by these observations was that spores produced on different varieties by the same culture varied in length. To study further the effect of host varieties on the length of conidia, several varieties were inoculated in the greenhouse with culture 22. Conidia from Wealthy, Yellow Transparent, Rome Beauty, Baldwin, and Northwestern Greening varieties were washed from the leaves and mounted in lactophenol solution for measuring. Two or more samples from different leaves were taken from each variety and 300 spores from each sample were measured. In each case, where spores were obtained from the ventral surface of the leaves, the different samples from the same variety were quite consistent. Spores taken from the dorsal surface of Baldwin leaves were shorter than those from the ventral surface. Two samples from each variety have been combined in figure 3, B, which shows the frequency curves obtained from the 600 spore measurements from each variety. These spores were all taken from the ventral surfaces of the leaves.

The modal length of conidia from Rome Beauty was 22 microns, representing 34.7 per cent of the spores. The mode for spores from Baldwin and Northwestern Greening was 20 microns, representing 40.4 and 43.2 per cent of the spores, respectively. The mode for Wealthy and Yellow Transparent was 18 microns, representing 40.4 and 46.3 per cent of the spores, respectively.

When culture 15 was used to infect Wealthy and Northwestern Greening the spore lengths were similar to those of culture 22, but 15 on Rome Beauty produced spores much shorter than those produced by 22 on this

variety. The modal conidial length of culture 22 on Rome Beauty was 22 microns and that of 15 was only 18 microns.

The present results indicate that different varieties varied the length of conidia of *Venturia inaequalis* when the varieties were infected by the same monoconidial culture. Moreover, on certain varieties different monoconidial cultures produced spores differing as much as 4 microns in their modal conidial length.

#### DISCUSSION

*Origin of Strains.* The major theories regarding the origin of physiologic forms of fungi are as follows: (a) adaptation, (b) mutation, and (c) hybridization.

No evidence has been found to indicate that adaptation to environment occasions more than temporary changes in monoconidial strains of *Venturia inaequalis*.

Mutations in parasitic fungi no doubt occur, and it is probable that new strains arise by this means. Sectoring has been noted in several monoconidial cultures of *Venturia inaequalis* but its nature has not been determined. Hansen and Smith (7) have presented proof that variations in *Botrytis cinerea* are due to the assortment of the nuclei and that this assortment is accomplished by the mechanisms of anastomosis and cell division.

The writer has noted hyphal fusions of *Venturia inaequalis* in culture. Besides the fusion of mycelial threads anastomosis of the germ tubes of conidia has been observed, often as many as 5 or 6 germ tubes being united. Frey (5) states that the cells of the mycelium are uninucleate and that each cell of the conidium is uninucleate. The nuclear behavior of *V. inaequalis* during anastomoses has not been studied, so the significance of the phenomenon in this fungus cannot be stated. However, until further study is made the possibility that variability may occur by this means must remain an open question.

Hybridization is known to occur in many fungi. The facts reported by Killian (11) and Frey (5) and confirmed by Wilson (18) show functional sex organs and suggest a true sexual process in *Venturia inaequalis*. Jones (8) reported the production of mature ascospores of *V. inaequalis* in culture after 5 months on oatmeal agar, but single-spore cultures were not used. Perithecia were initiated on several media by the writer when monoconidial cultures were used alone and in different combinations, but ascospores were not produced. It is not known whether *V. inaequalis* is homothallic or heterothallic. However, even though it be homothallic, it is possible that frequent matings between different strains might readily occur in nature and thus make possible the formation of new strains.

*Monoconidial Strain Variation in Venturia inaequalis.* Many workers [see bibliographies of Wallace (16) and Morris (13)] have listed certain

apple varieties as resistant or susceptible to apple scab. It is quite common to see trees of different varieties side by side, where one is heavily infected and the other is almost free of scab. Wallace (16) and others have pointed out many disagreements between reports of resistant and susceptible varieties. Aderhold (3) reported marked differences in the amount of disease on certain varieties in different seasons. Morris (13) states varieties relatively resistant in one locality may be quite susceptible in another.

It is quite likely that much of this disagreement is due to the prevalence of favorable environmental conditions for heavy infection when certain varieties happen to be at their most susceptible stage, while other varieties escape because they have not yet reached or have passed the more susceptible stage at the time of inoculation. However, it is also possible that different strains of *Venturia inaequalis* are responsible for some of the conflicting reports. The infection studies made by Wiesmann (17) as well as those of the writer show clearly that *V. inaequalis* is composed of several strains with different infection capabilities on certain apple varieties. The Yellow Transparent usually is considered relatively resistant to scab infection. The fact that this variety was entirely resistant to 7 of the 11 cultures with which it was inoculated during the present study may help to explain its freedom from scab infection in nature. The 4 cultures that did infect Yellow Transparent did so consistently and abundantly. When the 4 cultures that proved so virulent on Yellow Transparent were used to inoculate certain other varieties some of them were not able to infect at all. For example, Dudley and Missouri Pippin were entirely resistant to culture 19, which gave abundant infection on Yellow Transparent. Since most of the varieties studied by the writer were susceptible to several cultures, it seems likely that they would be found diseased in the orchard more often than a variety like Yellow Transparent, which can be infected by only certain cultures.

Much variation has been found between monoconidial isolation in their cultural characters as well as in their pathogenic capabilities on different apple varieties. However, no well-defined forms have been found that would correspond with the specialized forms of some other species of fungi. On the other hand, each monoconidial isolation of *Venturia inaequalis* made from naturally infected apples, either from the same locality or from widely separated regions, appears to differ in some of its cultural reactions and infection capabilities from any other isolation studied. Such findings indicate that *V. inaequalis* is not a homogeneous species or one composed of a few well-defined forms with definite cultural and pathogenic reactions but is one made up of many strains that differ in various degrees in their morphologic and physiologic characters.

## SUMMARY

Monoconidial cultures of *Venturia inaequalis*, isolated from 14 apple varieties obtained from Wisconsin, Oregon, Michigan, and New York, have been studied in relation to variability in culture, in pathogenicity, and in morphology.

Cultures differed from each other on nutrient media in the following colony characters: general appearance, margin of colony, size of colony, and abundance of conidia and perithecia produced. Cultures could easily be separated under standardized conditions by these characters.

The cultures also differed in their physiologic characters. The rate of growth has not been correlated with the locality or variety from which the cultures were isolated, since variations between isolations from the same variety and locality were as great as those between cultures from different varieties and States.

Although the optimal temperature for growth of all cultures was approximately 20° C. yet, at this temperature, certain cultures grew twice as rapidly as others.

Likewise, though the optimal pH for growth was between 4.8 and 5.8 under the conditions of these experiments for all cultures studied, with the exception of 30, yet, at this hydrogen-ion concentration, certain cultures grew much faster than others.

Culture 18 differed from cultures 21, 17, 19, 24, and 30 by better growth on solutions containing  $\text{Ca}(\text{NO}_3)_2$  as the source of nitrogen than those containing  $\text{KNO}_3$ .

Cultures varied in their tolerance to different concentrations of copper sulphate and Paris green in the nutrient on which the fungus was grown.

During the 3 years that these cultures were under observation some remained quite stable, while others, at times, produced variants in the form of sectors of the parent colonies. Such sectors differed from the respective parent colonies in general appearance, production of spores, and rate of growth.

The species of *Amelanchier*, *Aronia*, *Cotoneaster*, *Crataegus*, and *Sorbus* studied were not infected when inoculated with 6 cultures of *Venturia inaequalis*.

Of the 13 *Malus* species inoculated 9 were infected by 1 or more of the 6 cultures used and the other 4 showed no disease. Differences were found in the infection capabilities of the 6 cultures on the susceptible hosts.

Each of the 20 apple varieties inoculated was infected by one or more of the 9 cultures. Fifteen varieties were infected by each of the cultures with which they were inoculated, but the other 5 varieties were resistant to some cultures and susceptible to others under greenhouse con-

ditions. The cultures differed in their infection capabilities on the Yellow Transparent, McIntosh, Dudley, Missouri Pippin, and Hubbardston Non-such varieties.

Morphologic differences of certain cultures were found in the length and shape of the conidia produced in culture under uniform conditions. Five of the 7 cultures had spores of approximately the same length, while the spores of the remaining 2 were of different length. Conidial length also was influenced by the variety on which the spores were produced.

This study indicates that *Venturia inaequalis* is not a homogeneous species or one composed of a few well-defined forms with definite cultural and pathogenic reactions but is one made up of many strains that differ in various degrees in their morphologic and physiologic characters.

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# COMMELINA NUDIFLORA, A MONOCOTYLEDONOUS HOST OF A CELERY MOSAIC IN FLORIDA

S. P. DOOLITTLE AND F. L. WELLMAN<sup>1</sup>

(Accepted for publication February 17, 1933)

During the winter of 1928 a number of celery fields at Sanford, Florida, were severely damaged by a disease that produced a brown discoloration and shrivelling of the leaf stalks accompanied by a stunting of the plants. The losses from this trouble were confined to a limited area; but, where it occurred, yields were reduced from 10 to 80 per cent. The disease was brought to the attention of the senior writer late in the spring of 1928 but the crop had then been harvested in most of the badly damaged fields, and the symptoms on the remaining diseased plants had been complicated by the presence of blackheart and recent frost injury. It seemed possible that the trouble was of the mosaic type, but, as seen under the existing conditions, diagnosis was difficult.

In the spring of 1929 the disease again developed in severe form over the same area, and, at the request of the growers, several trips were made to Sanford during the latter part of the season. Investigations at this time proved that the disease spread rapidly in the field, that it was one of the mosaic group, and that it was readily transmitted by the melon aphid, *Aphis gossypii* Glover. During the summer of 1929 some further experiments were conducted in the greenhouses of the Department of Plant Pathology of the University of Wisconsin, at Madison, Wisconsin, and in the fall and winter of 1929-1930 a detailed study of the disease was begun by the senior writer at Sanford, the results of which are included in this paper (2). Since then the work has been continued by the junior writer and is still in progress.

## GEOGRAPHICAL DISTRIBUTION

The mosaic of celery, *Apium graveolens* L., here described has been found in all the important celery-producing sections of Florida and plants having apparently identical symptoms have also been found by the writers in California, Wisconsin, and Ohio and on market celery from New York. In 1922 Poole (9) described a mosaic of celery in New Jersey, and Elmer

<sup>1</sup> The early portion of this work was conducted at the Tomato Field Laboratory of the Florida Agricultural Experiment Station at Bradenton, Florida. Certain experiments also were conducted at the Department of Plant Pathology of the University of Wisconsin at Madison, Wisconsin. The writers are indebted to both institutions for the use of laboratory and greenhouse space in the course of this work.

The writers wish also to acknowledge the kindness of Mr. W. E. Stone and Mr. C. F. Stahl of the U. S. Bureau of Entomology in furnishing temporary office and laboratory facilities for the work at Sanford, Florida.

(4), in 1924, reported mosaic on celery in Iowa. It is probable, however, that they were dealing with a different virus. Harvey (6), in 1925, reported a mosaic on celery in Minnesota with leaf symptoms that appear to be the same as those found by the writers in Florida. No mention, however, was made of the discoloration and shrivelling of the petioles. Since 1924 the Plant Disease Reporter has contained reports of celery mosaic from States along the Atlantic Coast, as well as in Ohio, Indiana, Iowa, Minnesota, California, and Utah, but these limited reports do not permit any determination of the type of virus concerned.

#### LOSSES FROM MOSAIC

Celery mosaic occurred in the Sanford section for some years prior to 1928, and certain growers suffered occasional losses from the disease. In general, however, it attracted little notice. It seems to have become increasingly important in 1927 and, in the spring of 1928, caused losses from 10 to 80 per cent over an area of about 250 acres. In 1929, the damage was even more severe and some plantings were never harvested. Since the cost of production in the Sanford area is from \$300 to \$325 per acre, the monetary loss was great, even when the damage was confined to a comparatively limited acreage. Local estimates placed the loss at \$50,000 in 1928 and at approximately \$75,000 in 1929. In 1930 and 1931 the disease was much less severe; but, in 1932, although losses due to insects and fungous diseases complicated any estimate of losses from mosaic, it is safe to say that the damage was much greater than in any previous year.

#### SYMPTOMS

Healthy celery plants as seen in the field are of uniform height and of uniformly green color; but, when mosaic plants are present, an irregular, ragged, and readily noticeable effect is produced. The early symptoms of mosaic, as seen in Florida, consist of a pronounced outward and downward curling of the younger petioles, which gives the heart of the plant a characteristic open, flattened appearance (Fig. 1, A). The leaflets show a greenish yellowing along the veins, which later develops into whitish areas of irregular outline. The intervening green areas in the lamina remain green and this color intensifies with age (Fig. 2, A). In mature leaflets the green areas are somewhat thicker than the yellow portions. The leaflets are, therefore, slightly savoyed and crinkled. There is no filiformity or other deformity of the leaflets.

Mosaic celery plants appear stunted since the leaves tend to lose their upright habit but the length of the petioles is not greatly shortened, although their width and thickness are much diminished. As the disease pro-

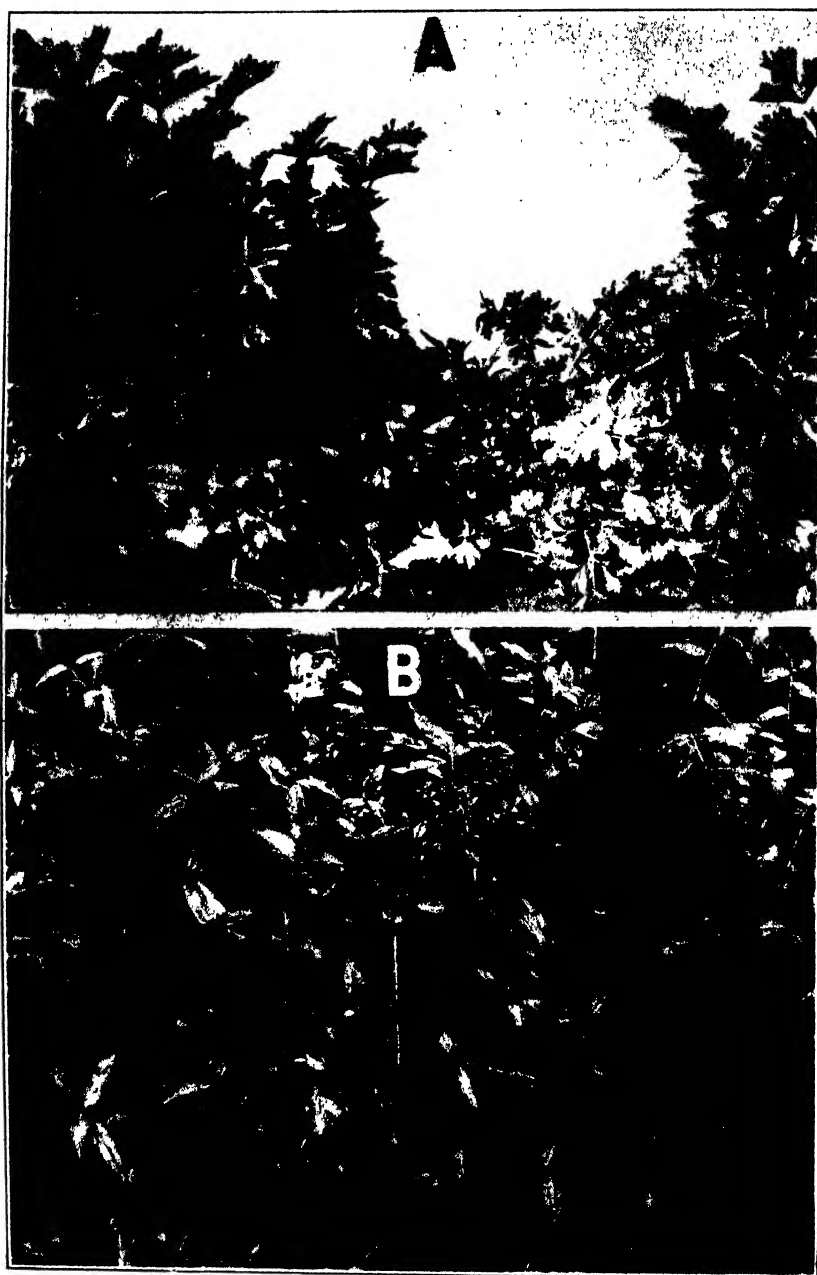


FIG. 1. A. Mosaic celery plant between healthy plants showing extreme downward curvature of petioles occurring in early stages of mosaic. B. Growth of mosaic *Commelina* occurring along border of celery field at Sanford, Fla.

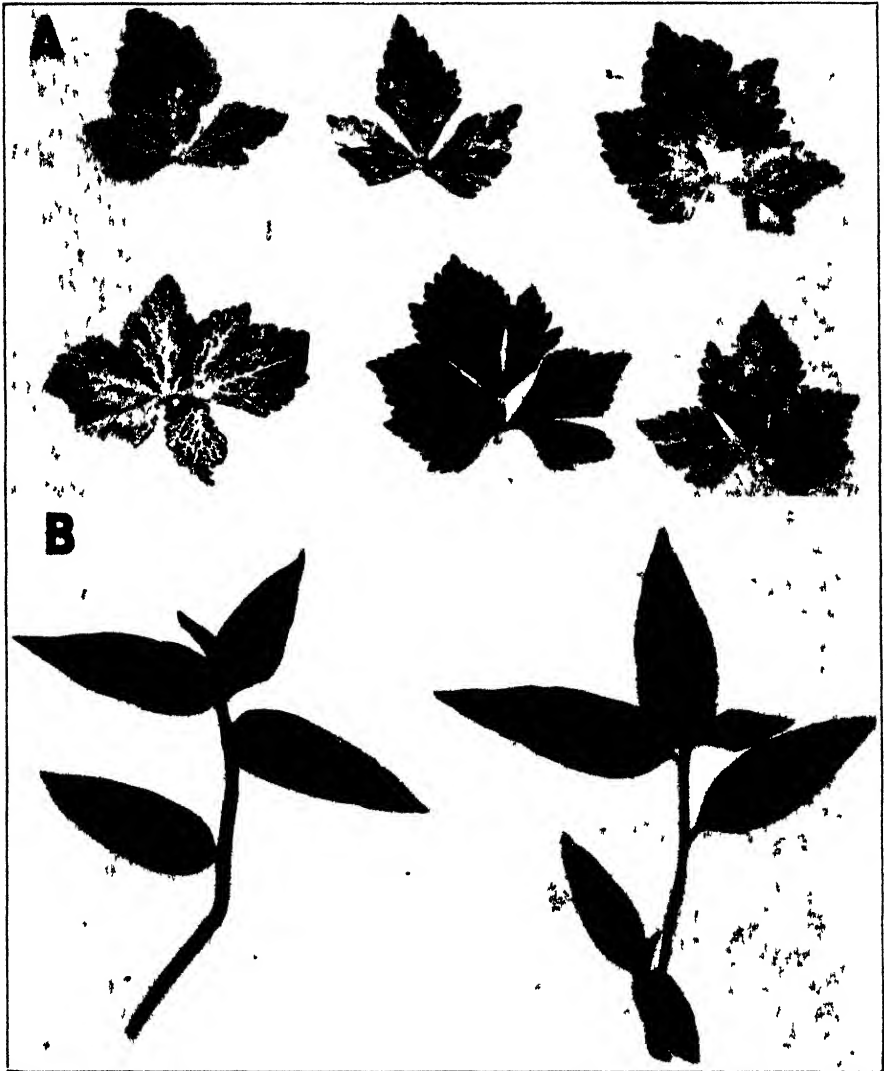


FIG. 2 A Mosaic celery leaflets showing pronounced mottling that occurs on young leaves. Healthy leaflet in lower center. B *Commelina nudiflora*. Plant at left shows typical mottling caused by mosaic, that at right is healthy.

gresses, buff and sunken translucent spots of irregular size and shape frequently develop on the petioles of mosaic plants. The vascular system of petioles thus affected is often discolored and, in severe cases, the petioles become brown and badly shrivelled (Fig. 3). Such plants have no commercial value.



FIG. 3. Mosaic celery plant showing discoloration, necrosis, and shrivelling of petioles. (Photographed by D. G. A. Kelbert, Florida Agricultural Experiment Station.)

Continued studies of celery mosaic, as it occurs in Florida, have made it increasingly evident that the symptoms of the mosaic we have been investigating are not those of the disease reported by Poole (9). He described mosaic plants as having a pronounced filiformity and other malformations of the leaflets, but without mottling or chlorosis. Necrosis of the petioles was not mentioned. Since these symptoms are the direct opposite of those found on mosaic plants in Florida, we, apparently, are dealing with a different virus from that described by Poole. Elmer described a celery mosaic (4) similar to that reported by Poole but mentioned a mottling of the foliage. Harvey (6), in his report of mosaic from Minnesota, figures celery leaves showing the type of mottling characteristic of the disease in Florida but he also figures a pronounced filiformity of the leaflets and makes no mention of stem necrosis. Foster and Weber (5), in 1924, were the first to describe celery mosaic in Florida, although they did not consider the petiole necrosis a symptom of mosaic.

#### DETERMINATION OF THE NATURE OF THE DISEASE

##### Aphid Inoculations

The field studies at Sanford in 1929 indicated that the disease was of the mosaic type and, since the melon aphid, *Aphis gossypii*, was abundant on celery plants in the fields where the disease occurred, the first experiments were conducted with this insect. Golden Self Blanching celery plants were secured from a section where no mosaic was known to occur and were transplanted to 5-inch pots, which were placed in a cheesecloth-covered house. After 3 weeks, the plants appeared healthy and aphids from healthy celery plants and from those showing symptoms of mosaic were transferred to plants from this stock under small cages. The aphids were transferred by means of a brush to small pieces of paper resting on a leaf of the healthy plant, and the insects soon moved to the celery foliage. Some aphids were collected from mosaic plants in the field but in most experiments aphids were taken from a known virus-free stock and colonized on mosaic celery plants that previously had been kept free from aphids. The results of these experiments, given in part in table 1, proved that the disease was readily transmitted by *A. gossypii*. A feeding period of 12 to 24 hours sufficed for the insects to become carriers of the virus and it was found that 5 aphids were able to produce infection, although 10 to 25 usually were placed on each plant. In all cases where infection occurred the symptoms were typical of those observed on naturally infected celery plants in the field.

Experiments were also conducted with the larvae of the celery leaf tyer (*Phlyctaenia rubigalis* Guenee), the garden flea hopper (*Halticus citri* Ashm.), the tarnished plant bug (*Lygus praetensis* L.), and the celery

TABLE 1.—*Transmission of celery mosaic by Aphis gossypii. (Experiments of March 13–October 2, 1929)*

Source of aphids	No. aphids per plant	No. plants inoculated	No. plants mosaic	Incubation period in days
Mosaic celery . . . . .	20–35	39	38	8
“ “ . . . . .	10	30	28	6
“ “ . . . . .	5	12	10	8
Healthy celery (check)* . . . . .	...	65	0	...

\* Check plants received same number of aphids as used on inoculated plants.

looper (*Autographa falcifera* Kirby), but these experiments gave negative results.

### Mechanical Inoculations

Attempts also were made to transmit the disease by mechanical means. In these inoculations the expressed juice of mosaic leaflets and petioles was pricked and rubbed into the leaflets and petioles of healthy plants or the freshly crushed mosaic tissue was inserted into wounds in the petioles or stem. These experiments proved that celery mosaic is transmissible by such mechanical inoculation, but the percentage of infection was much below that obtained with aphids. Table 2 shows results typical of those obtained by this method.

TABLE 2.—*Results of mechanical inoculation of celery with celery mosaic. (Experiments of April 10–October 25, 1929)*

Inoculum	Point of inoculation	No. plants inoculated	No. plants mosaic	Incubation period in days
Mosaic juice . . . . .	Petiole and leaflets	36	16	10
“ “ . . . . .	Petiole and stem	15	6	8
Mosaic-tissue fragment . . . . .	Stem	15	5	8
Healthy juice or tissue (check) . . . . .	As above	55	0	

### Cross-inoculation Experiments

Since the cucumber mosaic described by Doolittle (3) and listed by Johnson (7) as “cucumber virus 1” also is transmitted by *Aphis gossypii*, cross inoculations were made by means of *A. gossypii* from mosaic celery plants to cucumber and with cucumber mosaic to celery. Infection was secured in both cases and the same results were later obtained by means of mechanical inoculations. The incubation period of the celery virus on



the cucumber was approximately that of the cucumber virus on this host, and the symptoms, particularly in the later stages of the disease, were also much the same. It was found, however, that cucumber plants affected with celery mosaic often showed a yellow bleaching of the more mature leaves; and, in many instances, the early symptoms on the young leaves took the form of small, circular yellow areas scattered irregularly over the leaf. Neither of these symptoms is usually characteristic of the cucumber virus mentioned above, but similar symptoms do occasionally occur and no marked symptomatic differences were definitely established.

In the case of celery plants affected with cucumber mosaic, the incubation period was about the same as that of the celery mosaic occurring in the field. In the early stages of the disease the celery plants infected with the cucumber-mosaic virus showed a mottling of the leaves and a discoloration of the stem not readily distinguishable from those occurring on naturally infected celery plants. When infected with the cucumber virus, however, the celery plants retained a more erect habit of growth and occasionally showed a definite filiformity of the leaflets.

In 1929 it was also shown that *Aphis gossypii* could transmit celery mosaic to the tomato, *Lycopersicum esculentum* Will., the cultivated ground cherry, *Physalis pubescens* L., and tobacco, *Nicotiana tabacum* L. On these plants the symptoms were similar to those of cucumber mosaic on the same hosts but, as in the case of the cross inoculations on celery and cucumber, there were certain differences on occasional plants. In all the comparative cross inoculations with celery and cucumber mosaic, however, the general similarity in the type of symptoms produced was so marked that there was a question as to the significance of the prevalent minor differences, particularly, since the symptoms of cucumber mosaic also varied on the same host plants. It was evident, however, that the data then available were insufficient to establish the identity of the 2 viruses. Owing to circumstances the work on this phase of the problem was temporarily discontinued until the spring of 1931 when it was again taken up by Wellman, whose studies (10) have shown that the virus of the celery mosaic here described is probably not identical with that of the cucumber-mosaic virus used in these experiments.

#### Sources of Primary Infection

*Soil.* In the Sanford section, there was a general opinion that celery mosaic first appeared at the same points in the field each season. This had led to the belief that the causal agency was soil-born and, as a consequence, caused considerable alarm. In the fall of 1929 soil was taken from about mosaic celery plants and so stored as to keep the moisture content approximately equivalent to that of field soil. Seedling celery plants, grown in a

cheesecloth cage, were transplanted to 5-inch pots of this soil immediately after its removal from the field. The experiment was repeated at intervals of 30 and 60 days. Fifty plants were used in each series and were protected from insects by a cheesecloth shelter. The plants in each series were held for periods of 40 to 60 days, but no mosaic developed. In connection with the above experiments celery seedlings of various ages were removed from the soil and the roots clipped while immersed in the expressed juice of mosaic celery plants. The plants were then immediately transplanted to 5-inch pots and grown under cheesecloth for 3 to 4 weeks. Forty-six plants were thus inoculated, but no infection occurred. It appears, therefore, that celery mosaic is not soil-borne, and field observations have definitely supported this belief.

*Seed.* The question of the seed transmission of celery mosaic can only be definitely settled by growing seed collected from known mosaic plants, but up to the present such seed has not been available. Tests were conducted in 1929, however, with commercial seed planted in seed beds inclosed by a cheesecloth cage. The seed used was the same as that planted in the open on an adjacent farm where mosaic had occurred regularly for several years. Approximately 2,800 plants were grown under cheesecloth, but no evidence of mosaic appeared. About 2,000 of these plants were transplanted in the field, but no mosaic developed until so late in the season that it was evident that the infection must have come from other parts of the field. Seedlings from the same seed planted in the open showed no evidence of mosaic until long after transplanting and other seed beds in the vicinity appeared mosaic-free. Observations during the seasons of 1930 and 1931 confirmed these results, and, although further work is necessary before a definite statement can be made, it is probable that seed is of little importance as a source of primary infection.

*Wild Host Plants.* An arrangement was made for cooperative work at Sanford with the owners of two adjacent farms where celery mosaic had been particularly severe in 1927 and 1928. Through the kindness of these growers all of the soil and seed studies and most of the work on wild hosts was conducted on these farms. In order to secure a succession of cuttings from early to late in the season, the celery on these farms was planted in successive blocks with the rows running from north to south. The plantings were made approximately 7 to 10 days apart. The investigations were started early in November, 1929, at a time when the plantings were partially completed, the last block being set out November 25.

On one of the farms the earliest celery was transplanted on October 8 in a block at the extreme north end of the farm. This area was next to the highway on the north and was bordered on the east by the buildings and

lawn of the adjacent farm. By November 15 the celery in this early planting had developed a considerable amount of mosaic, nearly all of which occurred in the first 12 rows adjacent to the farm buildings on the east boundary. In the next block, planted 10 days later, mosaic was again confined to the same rows on the extreme east side of the field but occurred only in the north end of the planting. No mosaic occurred in the southern portion of this block nor in the third planting to the south. This definite localization of the mosaic infection was manifestly suggestive of the existence of a source of infection at definite points on the east side of the field.

From north to south, the vegetation along the east edge of this field consisted first of a Bermuda-grass lawn and then a group of trees and shrubs in which there was a thick weed growth. Beyond this was a barn where weeds also were abundant. Directly south of the barn were cultivated crops other than celery and here the narrow border between the farms was clean-cultivated. The celery plants in the rows next to the lawn were almost mosaic-free but the same rows showed a large amount of mosaic beginning at the point where the field was bordered by weeds. The mosaic plants continued down these rows until the cultivated border was reached. The amount of mosaic then decreased abruptly and, after a short distance, no more mosaic plants were found throughout the remainder of the field. Since the field itself was practically weedless, this occurrence of mosaic in restricted areas along its edge was definitely indicative of the existence of weed hosts. Further observations at other points in the vicinity confirmed the fact that the disease occurred only where there was an adjacent growth of weeds.

An examination of the weeds along the border of the field in question revealed 3 species showing what appeared to be possible symptoms of mosaic. One of these, known locally as Spanish needle, *Bidens leucantha* L., showed symptoms that appeared typical of mosaic, and a large number of cross inoculations were made to celery both by mechanical inoculations and by means of *Aphis gossypii*. No infection was secured, however, and attempts to transmit the suspected mosaic to *Bidens* itself also failed.

A small group of wild ground-cherry plants, *Physalis lagascae* R. & S., also were found showing symptoms of mosaic, and successful inoculations were made to celery and cucumber from this host by means of aphids. The symptoms produced were those of celery mosaic, hence there is no doubt that *Physalis lagascae* may carry the celery-mosaic virus. A continued survey, however, showed that this host was extremely rare in the vicinity of Sanford and no other mosaic plants of this species were found during the season. It was evident, therefore, that its presence did not account for the general infection in the field.

The third plant showing symptoms of a possible mosaic was the creeping dayflower, *Commelina nudiflora*, L., known in the Sanford section as wild Wandering Jew. This monocotyledonous weed is common about Sanford, particularly along ditches and in moist, shaded locations, where it often forms a dense mat (Fig. 1, B). It was abundant under the trees and around the buildings on the eastern edge of the field under study but did not occur to any extent in the lawn at the north nor to the south, where the border of the field was kept cultivated. It was evident, therefore, that celery mosaic had developed only where there were *Commelina* plants adjacent to the rows. The plants of *Commelina* that were suspected of being mosaic showed no marked dwarfing of the leaves nor shortening of the internodes. The leaves were, however, distinctly mottled with areas of greenish yellow, present either as rather large, irregular longitudinal patches or as roughly circular spots from 2 to 4 mm. in diameter. These yellow areas often extended across the veins and the mottling was not confined to the sharply defined longitudinal striping that characterizes the mosaic diseases reported on most monocotyledonous hosts (Fig. 2, B). *Commelina* is very sensitive to low temperature, and when the first observations were made in December, 1929, the plants were making little growth and the mottling was not so marked as that observed later on the foliage produced in February and March. It was noted that, while *Commelina* was abundant throughout the Sanford section, the mottled plants occurred most commonly in the immediate vicinity of fields where celery mosaic occurred each year. It was also found that *Aphis gossypii* was present on occasional plants of *Commelina*, including some that showed symptoms of mosaic.

As a result of these observations aphids that had been colonized on healthy cucumber plants were transferred to mottled and healthy plants of *Commelina* growing in cheesecloth cages. The aphids fed on this host fairly readily. At the end of 24 hours they were transferred to healthy celery plants by the method previously described. All plants used for inoculation were kept under cheesecloth cages. The inoculations proved that the mottled *Commelina* was affected with a mosaic that was transmissible by means of aphids and the symptoms produced were identical with those occurring on celery in the field. (Table 3.)

TABLE 3.—*Transmission of mosaic from Commelina nudiflora to celery by Aphis gossypii. (Experiments of January 14 to July 31, 1930)*

Source of aphids	Number aphids per plant	Number plants inoculated	Number plants mosaic
Mosaic <i>Commelina</i> .....	15-30	25	24
Healthy " (check) ....	15-30	19	0

Aphid inoculations were made also from mosaic *Commelina* to healthy plants of the same species and infection was secured on 10 out of 15 plants. Mechanical inoculations with the expressed juices of mosaic *Commelina* plants were not so effective as those in which aphids were used, the results in this respect being similar to those with this mosaic on celery. Infection was secured, however, in 7 out of 32 celery plants inoculated in this manner. Reciprocal cross inoculations were made from mosaic celery plants to healthy *Commelina* plants by means of aphids and infection was secured in 8 out of 12 plants. *Commelina* mosaic also was transmitted by aphids to the cucumber and the cultivated ground cherry. In both hosts the symptoms and those resulting from infection with the virus of celery mosaic were identical, and the plants showed the same minor variations from the normal type of cucurbit-mosaic symptoms noted on these hosts when inoculated from mosaic celery plants. A mosaic on *C. nudiflora* was reported by Kunkel (8) from Hawaii, in 1921, but no description was given of the symptoms. In 1931, Cook (1) also reported a mosaic on *C. longicaulis* Jacq. whose symptoms appear identical with those described above. Neither author reported any inoculation experiments.

#### DISCUSSION

It is evident that *Commelina nudiflora* is a host of celery mosaic and that *Aphis gossypii* can transmit the virus from the wild to the cultivated host. The occurrence of celery mosaic on *Commelina* is of unusual interest, since we have what appears to be the first definitely established case of a mosaic virus affecting both dicotyledonous and monocotyledonous host plants. The mosaic on *Commelina* is transmissible to at least 3 families of dicotyledonous plants, and, in view of its similarity to cucurbit mosaic, it possibly may be found eventually to have a similarly large host range. In view of the fact that the mosaics of corn, cane, wheat, and other grasses appear to be nontransmissible to dicotyledonous plants, it is perhaps significant that the symptoms of mosaic on the leaves of *Commelina* are distinctly different in type from those reported on these hosts.

From an economic standpoint the occurrence of celery mosaic on *Commelina* is of great importance, since this host is abundant in Florida and its presence probably accounts for nearly all the primary infection on celery in the Sanford area. Since *Aphis gossypii* is rather common on celery and also occurs on *Commelina*, an abundance of insect vectors is likely to be present throughout the season. It is probable, therefore, that the eradication of *Commelina* in the vicinity of celery fields offers the most effective means of controlling celery mosaic in Florida, and, owing to the intensive methods of cultivation employed in the Sanford section, it is the opinion of the growers that an adequate eradication of the wild host can be accom-

plished at relatively small expense. Experiments on the control of the disease by this method are being conducted and, while still in progress, they have indicated (10) that the eradication of *Commelina nudiflora* offers a practicable and effective means of controlling celery mosaic in Florida.

#### SUMMARY

During the past 4 years a mosaic disease of celery has caused serious losses in Florida and has also been found in other celery-growing sections in the United States. This mosaic appears to be distinct from those mosaics described by earlier workers, since, in addition to a definite mottling of the foliage and lack of malformation of the leaflets, it produces discoloration and shrivelling of the leaf stalks.

The disease may be transmitted by mechanical inoculation and also by *Aphis gossypii*, the latter being responsible for its rapid dissemination in the field. Celery mosaic has been transmitted to cucumber, tomato, tobacco, and the cultivated ground cherry. The symptoms on these hosts and on celery are much like those of the cucurbit mosaic described by Doolittle, but there are some differences and the two viruses are not thought to be identical.

The virus of celery mosaic does not appear to persist in the soil nor in the seed, but it has been found that the disease commonly occurs on a monocotyledonous host, *Commelina nudiflora*, and in one instance it has been found on *Physalis lagascae*. Field studies have shown that the primary infection of celery can be traced directly to mosaic *Commelina* plants near the borders of the fields and that *Aphis gossypii* acts as the vector from the wild to the cultivated host. It is believed that the eradication of *Commelina* is a practicable means of control for celery mosaic under Florida conditions.

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# CONTROL OF ELM LEAF SPOTS IN NURSERIES<sup>1</sup>

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(Accepted for publication March 11, 1933)

White elms (*Ulmus americana* L.), growing in nurseries in northern Illinois, were subjected to very severe injury by 3 kinds of leaf spot during the growing season of 1932. The fungi causing the spots were *Gnomonia ulmea* (Schw.) Thuem. (*Gloeosporium ulmeum* Miles), *Gloeosporium ulmicolum* Miles, and *Gloeosporium inconspicuum* Cavara,<sup>2</sup> the last being by far the most abundant and destructive in most of the nurseries seen. Accompanying the leaf spots was a severe tip blight of twigs, and when spotting was heavy severe defoliation occurred. This combination of injuries is, of course, very detrimental to the growth of young elms.

It has been suggested by Felt and Rankin<sup>3</sup> that "two or three applications of bordeaux at intervals of two weeks after the leaves are full grown should give reasonable control" of the elm leaf spots, but no data are available to show this to be a fact. Hence, it seems desirable to present some data obtained incidentally in 1932 while working on control measures for elm "wilt" fungi.

## SYMPTOMS OF ELM LEAF SPOTS

Though the symptoms accompanying attack by the first two fungi were described by Miles,<sup>4</sup> the following observations seem pertinent. Leaf spots due to *Gnomonia ulmea* are first visible as small yellow to whitish flecks that develop into small, black eruptions that are limited to the upper surfaces of the leaves. These often merge into small, coal black, stroma-like structures. In many cases these stromatic structures show very distinctly because they are surrounded by a narrow band of whitish, dead tissue. Infection is not limited to the leaf. Severe damage is frequently caused also by the blighting of young twig tips.

<sup>1</sup> The writer wishes to acknowledge his indebtedness to Mr. L. R. Tehon, Botanist of the Illinois State Natural History Survey, for suggesting the presentation of these data and for further suggestions and assistance during preparation of this article.

<sup>2</sup> This determination is somewhat uncertain. The fungus involved is not identifiable as *Septogloeum profusum* (E. & E.) Sacc., *S. ulmi* (Fr.) Bri. & Cav., *S. ulmeum* Davis, or *Myzospodium roseum* Dearn. & Barth. It does, however, conform macroscopically to the fragment of *Gloeosporium inconspicuum* Cav., exemplified in No. 350 of Briosi and Cavara's *I funghi parassiti della Piante coltivate ed Utile* and gives fungus characters in agreement with the description of that species.

<sup>3</sup> Felt, E. P., and W. H. Rankin. Insects and diseases of ornamental trees and shrubs. 507 pp. The Macmillan Company, New York. 1932. (p. 216.)

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Spots caused by *Gloeosporium ulmicolum* are visible on both the upper and lower leaf surfaces, the leaf tissue being killed in elongated spots along the midrib, veins, and leaf margins. Figure 1 shows the type of spot and the blight of growing tips caused by *Gloeosporium ulmicolum*.

The spots caused by *G. inconspicuum*, pictured in figure 2, differ considerably from the others. Characteristically, they are subcircular



FIG. 1. The leaf spot and twig blight caused by *Gloeosporium ulmicolum*.

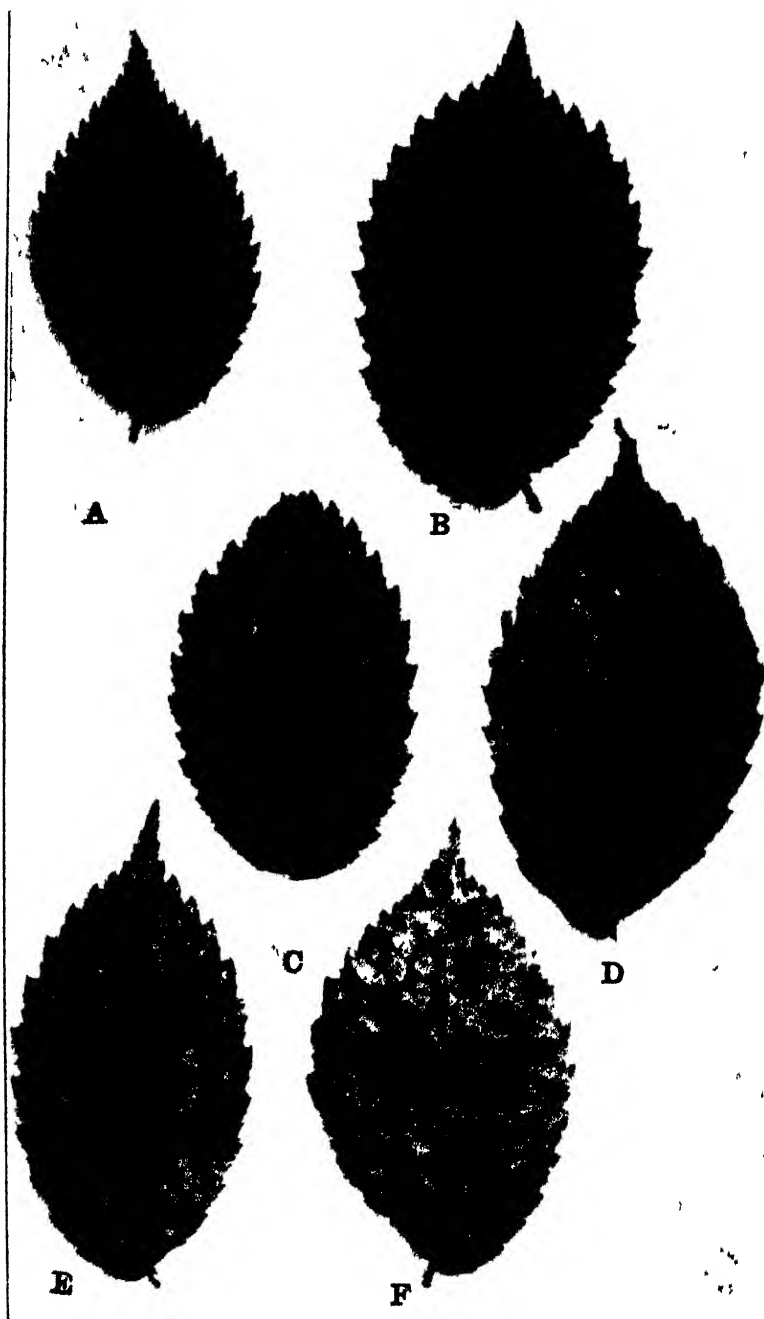


FIG. 2. Chart used as a scale in classifying leaves infected by *Gloeosporium inconspicuum*. See text for method of application.

and brown, with darker margins and dark centers in which the acervuli are found. There is no development of black,stromatic tissue; and the spots may be definitely limited, effuse, confluent, or irregularly extensive along veins. They extend through the leaves and are visible on both surfaces.

Though all 3 spots were prevalent in all plots during 1932, defoliation was most severe where the *Gloeosporium inconspicuum* spot predominated, and this spot seemed generally to be more productive of early and complete defoliation than the others.

#### EFFECT OF WEATHER

Clinton's<sup>5</sup> observations on *Gnomonia ulmea* in Connecticut in 1909 led him to state: "Infection seems to take place only early in the season, since the trees early denuded did not have their second crop of leaves attacked to any extent."

In the nurseries under our observation in northern Illinois in 1932, however, both the leaf spot caused by *Gnomonia ulmea* and the leaf spots caused by the *Gloeosporium* species showed progressive development. Initial infection appeared about May 20, and further infection developed through June, July, and August. Progressive development was, however, more noticeable in the *Gloeosporium* leaf spots than the *Gnomonia* spot.

In the course of the season it was observed that periods of 1 to 2 weeks elapsed, when less infection occurred than at other periods. This was shown especially by trees in control plots. On them, leaves on the lowest parts of branches would be heavily infected; on branch sections just beyond, few if any spots would be found; but, farther toward the tip, leaf spot would again be heavy. This rhythm of infection probably verifies the idea that weather plays an important part, but its occurrence on treated trees may also indicate that the fungicidal applications were made too far apart. When 4 weeks intervened between applications, there was an evident tendency toward leaf-spot infection to start on the young terminal leaves. This seems to indicate that much of the infection takes place while the leaves are very young.

#### MATERIALS

The materials used were Bordeaux mixture, Kolodust, Koloform, flotation-sulphur dust, and dry, wettable flotation sulphur. The Bordeaux mixture used was that prepared by the Corona Chemical Division of the Pittsburgh Plate Glass Company and sold under the trade name "Corona." At 8 pounds to 50 gallons of water it gives a 4-4-50 mixture. Kolodust is

<sup>5</sup>Clinton, G. P. Report of the Botanist for 1909 and 1910. 1. Notes on plant diseases in Connecticut. Bienn. Rpt. Conn. Agr. Exp. Sta. 33-34; 713-774. 1910. (p. 717.)

a proprietary dust of the Niagara Sprayer and Chemical Company. It is guaranteed to contain not less than 90 per cent sulphur and is ground finely enough to permit 98 per cent to pass through a 300-mesh screen. Koloform is a wettable bentonite sulphur, also prepared by the Niagara Sprayer and Chemical Company for use as a spray, 7.5 pounds to 50 gallons of water being the proportions. The flotation-sulphur dust here referred to is prepared by the Kopper's Products Company Laboratories. The flotation sulphur used as a spray, in the proportion of 5 pounds to 100 gallons of water, is also prepared by the Kopper's Products Company Laboratories and is known as Kopper's Dry Wettable Flotation Sulphur. It is guaranteed to contain not less than 80 per cent sulphur.

#### METHODS

In the spring of 1932 the writer, in cooperation with several nurseries in northern Illinois, started dust and spray experiments in 10 plots, 2 of which had been sprayed twice with 3-3-50 Bordeaux in 1931. All dust applications were made with a new hand duster, but the sprays were applied with such sprayers as were available at the various nurseries. In all cases care was used to get as thorough coverage and as good applications as possible under the circumstances. The treatments given and other details are shown in table 1.

All plots consisted of normal nursery rows, or parts of rows, of American elm. They were chosen and laid out with special reference to their suitability for testing the possible prevention of infection by the so-called "elm wilt" organisms by the application of fungicidal sprays. The control of elm leaf spots was an incidental result, and only such plots are reported here as could be adequately examined.

For the present purpose plots are divided into series, according to treatment. Series A, for example, designates the part of the plot not treated, while series B and C designate sections of the plot treated with fungicides.

#### STANDARDS FOR MEASURING CONTROL

As it was necessary to have some means of measuring the degree of infection, an arbitrary scale was made. Leaves representing a series of 6 classes of increasingly severe infection were chosen and these were arranged on cardboard in the form of charts, as shown in figures 2 and 3, so that direct comparison could be made between them and leaves from the trees. Class A included leaves having no spots to leaves having 5 spots; class B, leaves having 6 to 60 spots; class C, leaves with 61 to 180 spots; class D, leaves with 181 to 300 spots; class E, leaves with 301 to 420 spots; and

TABLE 1.—*Details concerning nursery elm plots and treatments given them in 1932*

Plot	Series	Number of trees	Size of trees in feet	Treatments			Date of readings
				Kind	Dates given	Lbs. pressure	
II	A	450	6-10	None	May 11 and 31 July 6	(Dust)	Aug. 13 Aug. 13
	B	450	6-10	Flotation sulphur Kolodust			
III <sup>a</sup>	A	600	10-16	None	May 18, June 9, July 13	200	Aug. 12
	B	600	10-16	4-4-50 Bordeaux			
IV	A	1000	5-7	None	May 18, June 9, July 13	200	Aug. 11
	B	1000	5-7	Koloform			
VI	A	528	2½ inches diameter	None	May 13, June 2, July 15	Hand pump “	Aug. 25 Aug. 25
	B C	528 528		4-4-50 Bordeaux Wettable sulphur			
VII	A	711	6	None	May 13, June 2, July 15	Hand pump “	Aug. 25 Aug. 25
	B C	474 474	6 6	4-4-50 Bordeaux Wettable sulphur			
VIII	A	800	4-5	None	June 16, July 8, July 22	(Dust)	Aug. 16
	B	800	4-5	Kolodust			
IX	A	379 539	5-6 Yearlings	None “	June 15, July 9, July 23	250	Aug. 18
	B	383 481	5-6 Yearlings	Koloform “			
X	A	400	8	None	June 16, July 2, July 29	(Dust)	Aug. 10
	B	400	8	Kolodust			

<sup>a</sup> Sprayed with 3-3-50 Bordeaux twice in 1931.

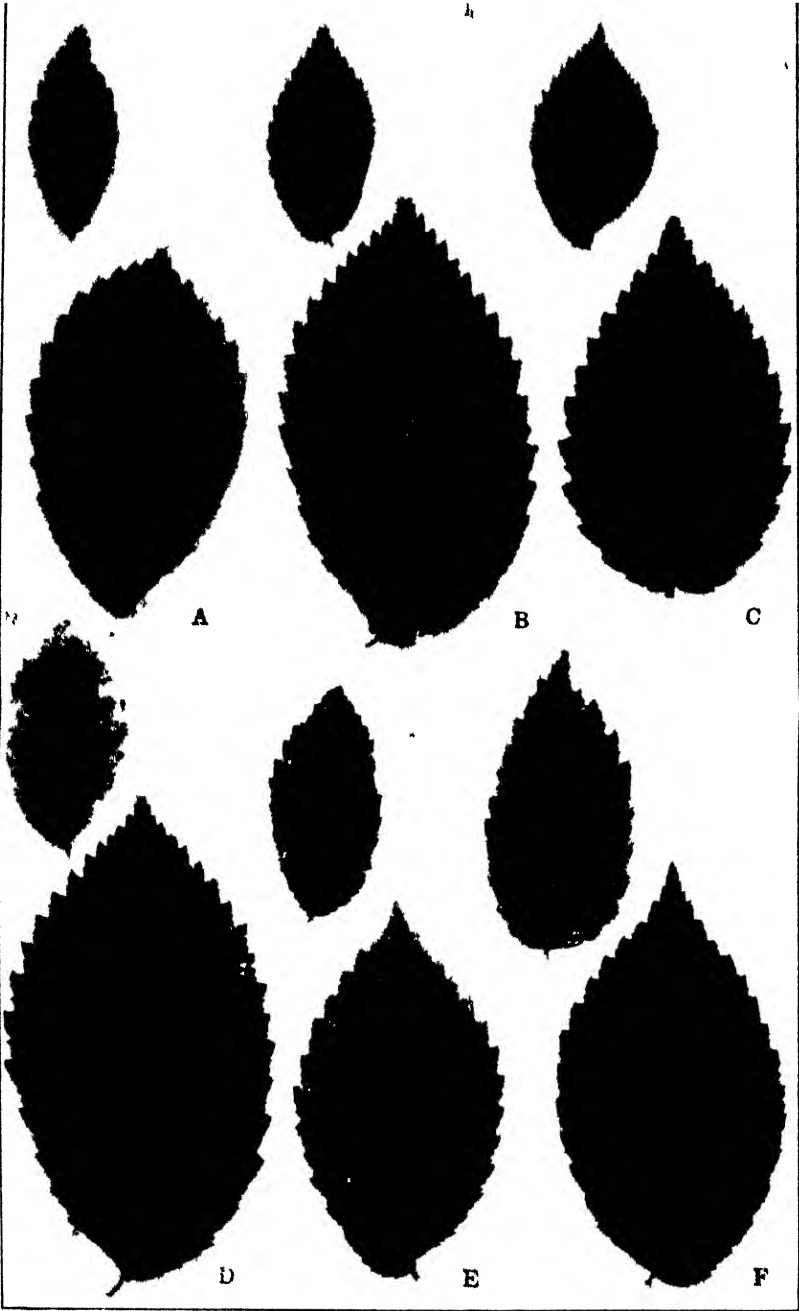


FIG 3 Chart used as a scale in classifying leaves infected by *Gnomonia ulmea*  
See text for method of application.

class F, leaves with 421 to 540 spots. These classes have the following midvalues:

A .....	2.5;	B .....	33;	C .....	120;
D .....	240;	E .....	360;	F .....	480.

By means of this scale, readings were made on every 10th tree, except in untreated series, where the occasional occurrence of trees too completely defoliated to read made it necessary to read the 9th or 11th tree. Readings were taken for all rows in plots VIII, IX, and X, and for  $\frac{2}{3}$  of the rows in plots II, III, IV, and VI. In making the readings, 50 leaves from each sample tree were compared individually with the standard leaves of the chart, and each leaf was allocated according to its appearance, but without an actual counting of spots, to the class to which it seems to belong.

It will be noted that the approved method of using uniform class ranges was not followed. Although the range in severity of infection was very great, some leaves having over 500 spots, the bulk of the leaves had in nontreated series less than half that number and in treated series less than a third. A large number of small-range classes would have made the task of taking readings an impossible one in the time at hand, while uniformly large classes would have failed to show remarkable differences in control exhibited within the lowest class. The series of classes and the scales based upon them are merely practical devices, quickly designed for an immediate use.

In determining the average leaf-spot infection for a given check or treated series, the number of leaves from that particular series allocated to each class was multiplied by the midvalue of the class. The products so obtained were then summed and the total was divided by the total number of leaves examined in that particular series. The quotient so obtained represents the average number of spots per leaf for the series.

Subtracting the average number of spots per leaf for treated series from the average number in the nontreated series gives a measure of the control obtained. This measure can be translated into a percentage expressing degree of control, by dividing it by the average number of spots in the check series. By means of the percentage, different treatments in the same plot and the same treatment in different plots can be compared as to effectiveness.

#### DATA AND COMPARISON OF RESULTS

A summary of the readings made in each plot is presented in table 2. The differences in distribution of leaves according to the degree of infection, as between nontreated and treated series, is very apparent. In classes C to F, which represents the heavier grades of infection, the nontreated series of each plot invariably show a much greater number of leaves than the treated series. This also is true in class B, with the exception of plot IV. But in class A, which includes the noninfected and the very lightly infected leaves, the treated series invariably show a leaf number several

times as great as the untreated series. This indicates in a general way the effectiveness of the treatments in reducing infection.

A more concrete expression of the effectiveness of the several treatments used is given in table 3, in which columns 3 and 4 are summaries of the detailed data given in table 2. It is evident that the results of treatment in any one plot cannot be compared directly with the results in any other plot, because of variations in conditions between plots due to geographic separation, soil differences, situation, and the like. Nevertheless, the results obtained in each plot with one or more treatments always show an appreciable reduction in the amount of infection in favor of the fungicide. This ranges from 40 per cent in plot IX, where Koloform was used, to 90 per cent in the dry, wettable flotation-sulphur series in plot VI, and there is a general effectiveness apparent in other plots of about 75 per cent.

TABLE 2.—*Distribution of grades of leaf infection in 8 experimental plots, comparing effects of various treatments vs. no treatment*

Plot	Series	Treatment	Number of leaves per class					
			2.5 spots per leaf	33 spots per leaf	120 spots per leaf	240 spots per leaf	360 spots per leaf	480 spots per leaf
II	A	None	500	903	126	55	15	1
	B	Flotation-sulphur dust	1337	250	11	2	0	0
III	A	None	557	1227	181	27	7	1
	B	Bordeaux	1553	277	17	3	0	0
IV	A	None	50	500	449	332	131	38
	B	Koloform	910	816	108	15	1	0
VI	A	None	162	176	77	43	26	16
	B	Bordeaux	391	89	13	6	1	0
	C	Dry wettable flotation sulphur	453	35	7	4	1	0
VII	A	None	539	357	60	25	13	6
	B	Bordeaux	830	156	12	2	0	0
	C	Dry wettable flotation sulphur	874	104	18	4	0	0
VIII	A	None	1696	257	25	22	0	0
	B	Kolodust	3775	211	13	1	0	0
IX	A	None	1437	346	35	20	10	2
	B	Koloform	1788	179	24	7	2	0
X	A	None	61	930	583	300	94	22
	B	Kolodust	1328	569	79	20	3	1



TABLE 3.—*Comparison of the average leaf-spot infection in treated and nontreated series in 8 experimental plots to show the effect of various fungicides in reducing the amount of spotting.*

Plot	Series	Treatment	Number of leaves examined	Average no. of spots per leaf	Difference in favor of fungicide	Degree of control obtained <sup>a</sup>
II	A	None	1600	41		
	B	Flotation-sulphur dust	1600	8	33	83
III	A	None	2000	37		
	B	Bordeaux	1850	9	28	76
IV	A	None	1500	141		
	B	Koloform	1850	25	116	82
VI	A	None	500	86		
	B	Bordeaux	500	14	72	84
	C	Dry wettable flotation sulphur	500	9	77	90
VII	A	None	1000	34		
	B	Bordeaux	1000	9	25	74
	C	Dry wettable flotation sulphur	1000	9	25	74
VIII	A	None	2000	11		
	B	Kolodust	4000	4	7	65
IX	A	None	1850	15		
	B	Koloform	2000	9	6	40
X	A	None	1990	114		
	B	Kolodust	2000	19	95	83

<sup>a</sup> Degree of control obtained is expressed as a percentage calculated from the ratio:

$$\frac{\text{Difference in favor of fungicide (column 5)}}{\text{Average number of spots per leaf in check (column 4)}} \times 100.$$

Bordeaux mixture and dry wettable flotation sulphur, used in comparable series in plots VI and VII, both gave good control but were not consistent in both plots, Bordeaux giving 74 per cent reduction of infection in plot VII and 84 per cent in plot VI as compared with 74 per cent and 90 per cent, respectively, obtained with the sulphur. As the natural infection in the check series was nearly 3 times as heavy in plot VI as in plot VII, these results might be taken to indicate that the sulphur spray is more effective than Bordeaux under conditions that produce heavier infections. The effectiveness of a sulphur spray also is indicated in plot IV, where the heaviest check infection encountered was reduced 82 per cent by Koloform. The effectiveness of sulphur dust against both heavy and light infections is indicated, also, in plots II, VIII, and X, Kolodust, giving a reduction of

infection of 83 per cent from the heavily infected check in plot X and 65 per cent from the lightly infected check in plot VIII and flotation sulphur dust, a reduction of 83 per cent from the moderately infected check in plot II.

The results appear to justify the statement that 4-4-50 Bordeaux, dry, wettable flotation-sulphur sprays and sulphur dusts will give good control.

#### DISCUSSION

Trees in plots IV, series B, sprayed with Koloform and plot X, series B, dusted with Kolodust, showed considerable contrast in comparison with the unprotected trees in plots IV A, and X A, respectively, because no defoliation occurred on the treated trees, while defoliation ranged from none to as much as 75 per cent on 50 per cent of the unprotected trees. The severe defoliation in the last-named plots was due largely to closeness of planting in the nursery and poor air circulation because of size of plantings and surrounding trees.

A close correlation was observed between the length of time moisture remained on the trees during the day and the intensity of leaf-spot infection. This was noticed especially in plot VIII, which was situated on a small elevation where there was a slight movement of air, even under very calm conditions. Several times dusting was postponed in this plot until about dusk, because of lack of dampness of the foliage. Dusting was tried about sunrise a few mornings and these trees were dry, even when there was heavy dew on weeds and grass below. Leaf-spot infection was light in this plot and the effect of the dust was not so marked. In contrast, plot IV and X were located on level land between blocks of elms, which prevented much air movement. Here the dew was retained until the sun caused its evaporation late in the mornings. The moisture present made it easily possible to get a good dust coverage of the wet leaves. Likewise, the conditions were favorable for maximum leaf-spot development.

One of the advantages obtained as a result of leaf-spot control was the easier recognition of wilt. There is a very close similarity in appearance between the tips of twigs and branches defoliated by leaf-spot fungi and those defoliated as a result of wilt. Where both kinds of disease are present on the same tree, it is difficult to differentiate between their symptoms in early stages. It was, therefore, an agreeable discovery to find it possible to eliminate most of the leaf-spot infection with fungicides. Such prevention makes it more easily possible to study the symptoms and control effects on the elm wilts.

Whether or not it is practical to apply fungicides for the control of elm leaf spot will have to be determined by the individual nurseryman and will depend upon the prevalence of the fungus in his particular nursery. Control of leaf mites also may be effected to some degree with the same application of fungicides. The foliage of trees sprayed with Bordeaux or Kop-

per's dry, wettable flotation sulphur in plot VII and with Kopper's flotation sulphur in plot II was found much freer from leaf mites than the unprotected checks in these plots. The intensity of infection of elm trees with the leaf-spot fungus depends primarily upon favorable environmental conditions, since the spores are present wherever the trees are grown. Conditions conducive to an environment favorable for an epiphytotic of leaf spot are, first, sufficient rains or dew and second, trees planted close together in the rows. This environment may be amplified by having many rows together in large blocks and in a sheltered location. General observations and results of these experiments indicate that it is advisable to so modify the cultural practices in use by nurseries growing elms as to limit the size of the blocks or to alternate the tree species more often in the blocks. Especially, it seems advisable to set the trees in strips of not more than 5 or 6 rows together, leaving between these strips spaces wide enough to permit passage of a power-spray rig.

There were no indications that the fungicides used were toxic to the foliage of the elms. For trees ranging from 5 to 8 feet in height, about 1 pound of sulphur dust was found sufficient to make one application to 40 trees, while, in the case of spray material, 1 gallon gave satisfactory coverage to 5 to 17 trees, depending upon the size of the tree and the amount of foliage. For yearlings to trees 5 feet high about 1 gallon is required for every 17 trees, while trees 12 to 16 feet high require about 1 gallon for every 5 trees.

#### SUMMARY

Fungicides used, in 1932, in 8 experimental plots of elm-nursery stock to control wilts were found to have a very considerable additional effect in reducing infection by 3 common leaf-spot fungi, *Gnomonia ulmea*, *Gloeosporium ulmicolum*, and *Glocosporium inconspicuum*.

The fungicides used 4-4-50 Bordeaux mixture, Koloform, dry, wettable flotation-sulphur spray, Kolodust, and flotation-sulphur dust were found to be about equally effective.

Three applications of the above fungicides reduced elm-leaf-spot infection by as much as 40 to 90 per cent, depending on the location of the plot and the amount of infection that developed.

The data presented show that 3 applications of any of these fungicides will hold leaf-spot infection under control and prevent defoliation. However, if 4 or 5 dust or spray applications could have been given over the same period of time a more constant coverage might have reduced the infection still more.

BOTANICAL SECTION,

ILLINOIS STATE NATURAL HISTORY SURVEY,

URBANA, ILLINOIS.

## PHYTOPATHOLOGICAL NOTES

*A Plant Inoculator.*<sup>1</sup>—The need of a device for rapidly making uniform puncture inoculations was felt in connection with a study on resistance to bacterial wilt of corn, which required inoculation of many plants in a relatively short time. The usual method of puncture inoculation by means of an ordinary needle seemed inadequate for that purpose because progress was too slow and inoculations thus made were not uniform. Use of a common hypodermic syringe for inoculation of corn plants was found unsatisfactory because the needle plugged very frequently, the plunger was slow and inconvenient to operate, and ejection of small, uniform amounts of inoculum was difficult. The instrument here described was constructed and so operated as to make puncture inoculations easy, rapid, and uniform.

The main features of this instrument are the side opening of the needle, which prevents plugging; ejection of the inoculum by gravity; and regulation of inoculum outflow by means of an air valve. These features, previously employed in various ways, were combined in an instrument of the capacity and strength adapted to the purpose.

This plant inoculator consists of 3 major parts: needle, barrel, and air valve.

The needle is made from an ordinary hypodermic, stainless-steel, screw needle by plugging the end and making a lateral opening near the tapering end (Fig. 1, A, a). This pointed end of the needle is made very smooth and sharp to avoid cutting the plant tissue into small pieces that might occasionally cause plugging of the needle. A screw adapter (Fig. 1, A, b) is provided for attaching the needle to the barrel.

The barrel is made of a nickel-plated metal jacket (Fig. 1, A, c), with a long, wide lateral incision to permit observation of the inoculum; an inoculum cylinder of glass, inserted in the jacket (Fig. 1, A, d); and a screw cap (Fig. 1, A, e) attached to the jacket.

The air valve (Fig. 1, A, f) consists of a hollow cylindrical screw, screwed into a tube attached to the cap. A small hole is drilled through both screw and tube through which air is admitted or excluded at will.

Thick rubber washers placed at the needle end of the barrel and inside the cap make the barrel air-tight, and the glass cylinder, made slightly longer than the jacket, is pressed against the washers by screwing the cap down tight.

This inoculator was used in the following manner: It was filled with the bacterial suspension aseptically by sucking on the open-air valve and draw-

<sup>1</sup> Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

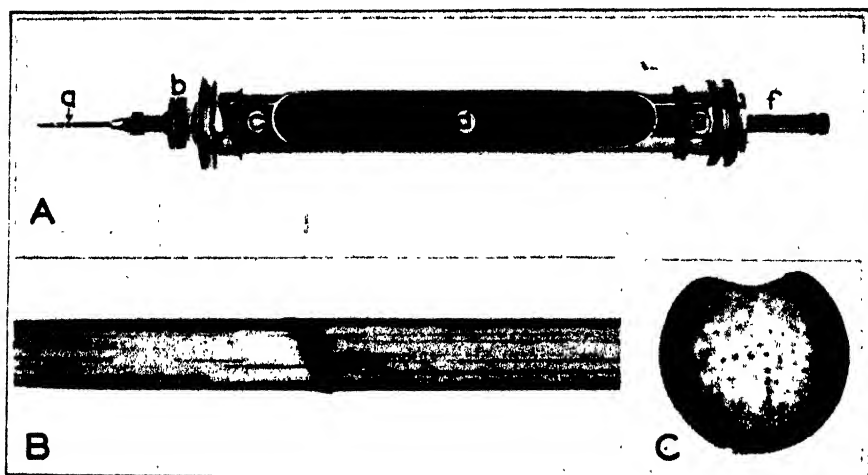


FIG. 1. A. Side view of the plant inoculator: a, hollow needle with a lateral opening; b, screw adapter; c, nickel-plated metal jacket; d, glass cylinder inserted into jacket; e, screw cap; f, air valve. B. Longitudinal section of corn stem inoculated with mixture of wilt bacteria and India ink by means of inoculator. The long, parallel black lines represent vessels filled with bacteria and India ink. The thick black line in middle represents cavity made by needle. C. Cross section of another corn stem inoculated with same mixture. Two punctures were made through stalk, one above the other at right angles. The black dots represent cut ends of vessels filled with bacteria and ink.

ing the liquid through the needle. After the inoculator was filled the hole of the air valve was closed to prevent the liquid from escaping through the needle. The filled instrument in this condition was carried from place to place in the field without spreading the inoculum. Rate of flow of the drops of inoculum through the hole of the needle was regulated as desired by adjusting the flow of air through the air valve. The most convenient rate of flow for inoculating corn plants was 1 drop per 2 seconds.

Inoculation of a corn plant was made by thrusting the inoculator needle through the stem and withdrawing it after a second. When the tip of the needle touched the surface of the stem a drop of the inoculum ran down along the needle and was deposited at the place of contact. At the time of the withdrawal of the needle the drop was taken into the needle cavity and the vessels of the plant, probably by negative pressure and capillarity. Penetration of the inoculum into the tissue of the plant immediately after inoculation was tested in a few cases by filling the inoculator with a mixture of wilt bacteria and India ink. The presence of the India-ink particles among the bacteria facilitated tracing the path of the bacteria before and after cutting the stem open. The black suspension was observed to penetrate the vessels of the vascular tissue for more than 4 inches above and below the puncture a few seconds after inoculation (Fig. 1, B and C).

The inoculator may be made to almost any size, according to the need. In inoculating corn plants 6 weeks old or older, a 16-gauge needle  $\frac{3}{4}$  in. long and a barrel of nearly 100 cc. capacity were used. More than 300 plants were inoculated at one filling.

The instrument was taken apart easily for cleaning. It was sterilized before using by boiling in water or by washing in alcohol and rinsing in sterile water.

The efficiency of this inoculator was illustrated by the fact that in the summer of 1933, 4 men, making two punctures per plant, inoculated in 4 days more than 100,000 plants.—S. S. IVANOFF, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

*Losses from Potato Late Blight in 1885 and 1886.*—Detailed estimates of losses from potato late blight (caused by *Phytophthora infestans* (Mont.) de Bary) for the year 1885 and 1886 have just become available to the Plant Disease Survey through the courtesy of two collaborators, S. H. Essary and C. D. Sherbakoff. The value of the material obviously warrants its publication, even though delayed. Some statement of the history of this unusual material seems also to be relevant.

### *The Manuscript*

In February, 1933, Essary found among some old papers in the Botanical Department of the University of Tennessee a roll containing 3 manuscripts. One was a typescript of the text of Marshall Ward's paper on *Phytophthora infestans*.<sup>1</sup> The second was entitled "Early History of the Potato Rot in the United States" and is an excellent summary of the history of late blight in the United States, obviously taken from the reports of the Commissioner of Patents. A pencil footnote indicates that this is by E. F. Smith, and it may well have been the result of one of the first assignments of work in the Department of Agriculture to the then (1886) newly appointed assistant. The final paragraph was begun with a typewriter and concluded with pen and ink.

"Since 1850, the ups and downs of potato growing have been many. Some years, and in many sections for a series of years, there has been very little rot. In exceptional years it has prevailed almost everywhere. The last great rot period was from 1884 to 1886. *The per cent of the losses from the disease in 1885 and 1886 is presented in the following tabular statement. This table is compiled from individual reports made in reply to a circular of inquiry sent out by the Department.*"

<sup>1</sup> Ward, H. Marshall. Illustrations of the structure and life-history of *Phytophthora infestans*, the fungus causing the potato disease. Quart. Jour. Micros. Sci. [London] 27: 413-425. 1887.



by States and Counties the Estimated Loss from Potato Rot in the United States in 1885 and 1886."

### *Estimated Losses*

The figures on the accompanying maps, figure 1, A and B, are in the "Average Per Cent of Loss" for each State as given in the manuscript tables. They are simple averages of the estimated per cent of loss for the counties reporting. The number of reports was surprisingly large; in 1885 there were nearly 2,500 replies (page 122<sup>2</sup>). Michigan sent more than 200 each year and New York almost as many. Ohio, Wisconsin, and Iowa sent over 100 reports each year. These figures are admittedly mere estimates and undoubtedly estimated losses here attributed to late blight may be due in part to other causes. The distribution of the losses, however, confirms the belief of the original compilers that the chief factor in the losses was the "rot" due to *Phytophthora infestans*.

A diagram showing the estimated losses in bushels and value in 1885 for 6 States is appended to the Report of the Mycological Section for 1886.<sup>2</sup> The figures for the other States and those for 1886 were apparently never published. The available information can leave no doubt that in 1885 there were serious losses from potato late blight throughout the Northeastern United States. In 1886 the disease was, in general, much less severe than in 1885, but there were very large losses in Rhode Island and Connecticut.—NEIL E. STEVENS, Bureau of Plant Industry, Washington, D. C.

*Pod Deformation of Mosaic-infected Peas.*—In a pea-disease survey, Linford<sup>1</sup> in 1928 reported the occurrence of deformed pods on mosaic plants of *Pisum sativum*. In California pod damage has become of considerable concern to the grower of summer and fall peas. When the plant becomes infected with the virus prior to the completion of pod development, the pods become markedly distorted (Fig. 1, B-C). The ovary wall assumes a rough, ridged, wrinkled condition and, as a result of these corrugations, is badly deformed and somewhat dwarfed. The ridges usually appear dark green in color. Pods resulting from blossoms borne on a badly diseased vine may become so badly twisted or curled as to be hardly recognizable as pods (Fig. 1, D-F). Such pods remain severely stunted and may produce no seed. Pods may reach all conditions of maturity on mosaic plants, ranging from the aborted condition just described to that seen in figure 1, B-C, where the seeds have matured.

<sup>2</sup> Scribner, F. Lamson. Report of the mycological section. In Report of the Commissioner, U. S. Dept. Agr. 1886: 95-138. 1887.

<sup>1</sup> Linford, M. B. Pea diseases in the United States in 1928. U. S. Dept. Agr. Bur. Pl. Indus. Suppl. 67. 1929.





FIG. 1. Pod deformation produced by the pea-mosaic virus on *Pisum sativum*. A. Pod of a healthy plant of the Hundredfold variety. B-C. Disfiguration and roughness of pods from Hundredfold plants infected with mosaic late in their development. D-F. Badly distorted pods that have reached maximum development on vines of the Stratagem variety infected early with mosaic.

The interior of a mosaic pod also presents a roughened appearance. The knobby ridges of the exterior are replaced by creased indentations of the membranous lining of the pod cavity, and the depressions of the exterior correspond to the irregular knobby areas of the inner pod wall. The seeds in diseased pods sometimes appear smaller and yellower than normal. Upon holding an opened mosaic pod to the light, the zones responsible for the raised irregularities of the outer surface appear dark green in contrast to the green of the rest of the pod.

The foliage symptoms of pea mosaic in California agree fairly well with those Doolittle and Jones<sup>2</sup> described for Wisconsin; in fact, the writer obtained malformed pods at Madison in the fall of 1930 on Perfection peas inoculated with mosaic. Aphis from mosaic plants were employed in the transmission of the virus, and, under greenhouse conditions, first symptoms were expressed on the foliage after an incubation period of 6 to 9 days. In California the foliage symptoms range from a simple mottling or vein clearing of the foliage to a mottling accompanied by irregular necrotic specks or spots up to 2 or 3 mm. in extent and an extreme twisting, curling, and stunting of the growth, which, in advanced stages, becomes rosetted. The petioles, tendrils, and even stems may be distorted in very severe cases. Whether these differences are due to strains of the virus of greater or less virulence or to a virus complex, remains to be determined. At any rate, the disfigured pods occur on mosaic plants in California regardless of the particular type of leaf symptoms expressed.

As Linford noted, the loss from mosaic is sustained in two ways. First, through a decreased yield as a result of the stunting and reduced productiveness of the infected plant and second, through unmarketability of the deformed product. The latter is the most conspicuous of the two types of loss, for it becomes necessary to cull out the distorted pods on the sorting belts. The culls from this source alone amount to 10 per cent and more of the picked crop in some instances.—WILLIAM C. SNYDER, Division of Plant Pathology, University of California, Berkeley, California.

*A Tropic Response in Germ Tubes of Urediospores of Puccinia graminis tritici.*—During a recent microscopic examination of the surface of wheat leaves inoculated with urediospores of *Puccinia graminis tritici* Eriks. and Henn., it was noticed that many of the germ tubes of these spores grew directly across the leaves, that is, in a direction at right angles to the veins (Fig. 1). Further examination has shown that the germ tubes of urediospores have a well-marked tendency to grow transversely on wheat leaves. A brief consideration of the stomatal arrangement on the leaf will show that this

<sup>2</sup> Doolittle, S. P., and F. R. Jones. The mosaic disease in the garden pea and other legumes. *Phytopath.* 15: 763-772. 1925.

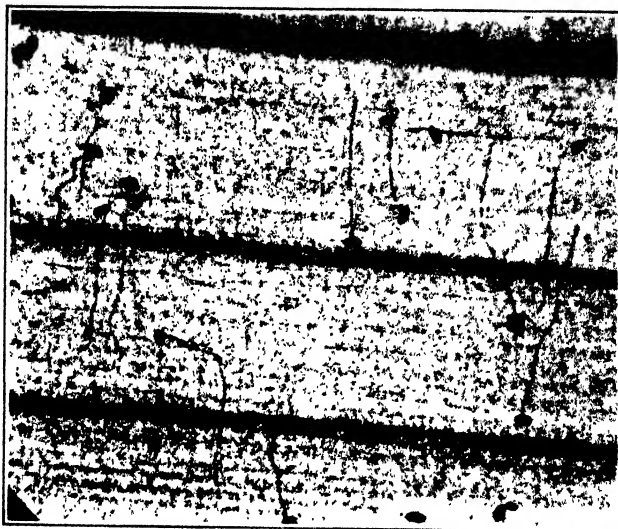


FIG. 1. Germinating urediospores of *Puccinia graminis tritici*, showing germ tubes elongating transversely on a wheat leaf.

manner of germ-tube growth is of considerable advantage to the rust as a means of securing a high proportion of penetrations. The stomata are arranged in longitudinal rows on the leaf; but the stomata of 2 adjacent rows are rarely opposite one another. Hence, a germ tube growing directly across a leaf is bound to encounter a stoma sooner or later, if it continues to elongate in a straight line.

Although this behavior of the germ tubes is known to have been observed previously by at least one other investigator,<sup>1</sup> no published reference to it is known to the writer. Plowright<sup>2</sup> and Stakman,<sup>3</sup> however, present drawings that show germ tubes of *P. graminis* Pers. entering stomata laterally.

In the present study a number of attempts have been made to determine the nature of the stimulus responsible for this directional growth of these germ tubes. The growth of the uredial germ tubes of *Puccinia graminis tritici* was observed on leaves of *Helianthus annuus* and a *Freesia* sp. On leaves of both species the germ tubes grew at random in all directions, except on the veins and midribs where the growth was generally straight across, as on wheat leaves. An examination of the epidermal structure of

<sup>1</sup> Dr. Margaret Newton, working under Prof. W. P. Fraser at McGill University, in 1919.

<sup>2</sup> Plowright, C. B. A monograph of the British Uredineae and Ustilagineae . . . 347 pp. K. Paul, Trench & Co., London. 1889.

<sup>3</sup> Stakman, E. C. A study in cereal rusts. Physiological races. Minn. Agr. Expt. Sta. Bul. 138. 1914.

the veins and midribs of these plants reveals a marked similarity to that of the wheat leaf. In both the cells are long and narrow with the lateral cell walls running parallel to one another, and at each cell wall there is a slight depression in the leaf surface. On other parts of *Helianthus* leaves, where the germ tubes grow at random in all directions, there is less similarity to the epidermal structure of the wheat leaf, the epidermal cells being rather irregularly arranged and the stomata more or less randomly distributed; and on *Freesia* leaves, the stomata are arranged in parallel rows, but the lateral cell walls are not straight as in wheat leaves but deeply sinuate.

It seems, therefore, that there is a correlation between the epidermal structure and the direction of germ-tube elongation on the leaf surface. There appears to be a well-marked tendency for germ tubes to cross cell walls at right angles. It is probable that the causal stimulus is of a thigmotropic nature, especially in view of the fact that light and geotropism were eliminated as causal agencies in the experiments. The behavior of the germ tubes was the same in diffuse light as in darkness, and the position of the leaf, whether vertical or horizontal, had no influence on the direction of growth.

There is, indeed, visible evidence of an interaction between the cell wall and the germ tube crossing it. Over each cell wall the germ tube produces a slight swelling containing an aggregation of protoplasm, as if a rudimentary appressorium had been formed by the germ tube before it crossed the cell wall. In preparations stained with cotton blue, these swellings resemble dark blue knots superimposed on the cell walls.

If the growth-directing stimulus is a thigmotropic one resulting from the contact of the germ tube with the finely grooved surface of the wheat leaf, it is apparently rather delicate, as attempts to direct the growth of germ tubes by means of artificially grooved glass, collodion, or wood surfaces have proved unsuccessful.

This apparent response to the parallel arrangement of cell walls is not confined to graminaceous rusts, as the germ tubes of urediospores of *Puccinia helianthi* (Schw.) show a marked tendency to cross the veins of sunflower leaves at right angles and to grow transversely on wheat leaves. This tendency was, however, not detected in the germ tubes of urediniospores of *Coleosporium solidaginis* Thm.—THORVALDUR JOHNSON, Dominion Rust Research Laboratory, Winnipeg, Canada.

*An Improved Method of Bleaching and Clearing Leaves.*—In the course of determining the detail structure of vein terminations in leaves and petals<sup>1</sup> it was found necessary to bleach and clear the material so that the

<sup>1</sup> Strain, R. W. A study of vein endings in leaves. *Midland Naturalist* 14: 367-375. 1933.

epidermal cells and mesophyll were sufficiently transparent to allow study and drawings to be made of the vein endings. The reagents commonly used for this purpose did not prove entirely satisfactory. Frequently, fresh green leaves were easily cleared in a given solution, while leaves of the same species, when dry, or when collected in autumn after the chlorophyll had begun to disintegrate, were very difficult to clear or could not be cleared at all so that they could be accurately studied. Lactic acid was a reagent of this type.

Even though fresh material, as a whole, was more easily bleached and cleared than dry material, it was necessary, in order to study a larger number of species, genera, and families than were available in a living condition, to use herbarium specimens. The dried material introduced new difficulties in clearing, for many of the herbarium specimens could not be satisfactorily cleared in lactic acid, although it was heated. This resulted in the use of another substance, a mixture of lactic acid, phenol, and glycerine (Aumann's solution). This gave better results than lactic acid alone. It made the leaves firm and easy to handle, while the lactic acid rendered them soft and somewhat gelatinous. However, with this solution, many of the leaves could not be properly cleared. With continued heating in a boiling solution of this mixture, the leaves turned brown and no subsequent treatment improved their condition.

Another method was sought to clear those leaves that could not be cleared by any method used thus far. Quick and Patty<sup>2</sup> used, with good success, a mixture of commercial sodium hypochlorite and sodium hydroxide as a bleaching agent. This solution did not prove entirely successful with the specimens under investigation.

Since the bleaching in the method of Quick and Patty was indirectly due to the chlorine, the writer thought the introduction of nascent chlorine into water would give quicker and better results. Chlorine was prepared by the action of concentrated hydrochloric acid on potassium chlorate and used with fair results. One disadvantage was that, unless the specimens were used within a day or two, they disintegrated. It was also noted that in a few cases specimens that appeared to be bleached, turned brown when placed in the lactic acid-phenol-glycerine mixture.

The writer decided that, since alcohol is often successfully used to extract chlorophyll from leaves, the introduction of chlorine into alcohol might prove satisfactory. This was done with the best results thus far obtained. The time required for clearing the leaves varied from a few minutes to two hours, depending upon the type, size, and condition of the leaves, and the amount of chlorine introduced. Two advantages in using

<sup>2</sup> Quick, C. R., and F. A. Patty. A convenient method of bleaching and clearing leaves. *Phytopath.* 22: 925-926. 1932.

alcohol instead of water were, first, that the specimens were bleached more effectively and, second, they could be left for several days in the solution with no serious effect. It is to be understood that even with this method the leaves were afterwards heated in the lactic acid-phenol-glycerine solution to clear them completely. In all probability any leaf regardless of age or whether it is fresh or herbarium material can be cleared so well by this method that one can accurately study vein endings, epidermal or palisade cells, stomata, and crystals.—ROY W. STRAIN, University of Michigan, Ann Arbor, Mich.

*A Species of Sphaceloma on Avocado.*—Laboratory and field studies have shown that the *Sphaceloma* on avocado, *Persea americana* Mill., is an undescribed species.<sup>1</sup> The specific name *S. perseae* is, therefore, proposed for it and the following description given:

***Sphaceloma perseae*, n. sp.**

Lesions generally brown to almost black, up to 3 mm. in diam.; conidial fructifications, acervuli at first, with further development constituting sporodochia or more or less free conidiophore tufts, scattered to effuse, in mass, dark olive or light brownish olive,  $25-70 \pm \mu$  in length; conidiophores at first 1-2-celled, often about  $12 \mu$  high by  $2-7 \mu$  at base, tapering, or acute, to truncate at apex, arising from hyaline intra-epidermal hyphae or from prosenchymatous stroma, palisaded, on rupturing the epidermis increasing in length by continued growth or by conidia remaining *in situ* and developing as a part of the conidiophores, often 25 to  $50 \mu$  long, reaching  $100 \mu$ , more or less divergent, continuous to several septate, straight or geniculate, usually simple, sometimes denticulate, apical region often paler than rest of conidiophore; conidia acrogenous or pleurogenous, at times several produced from the same point, hyaline or colored, clear or granular, spherical to cylindrical,  $2-30 \times 2-5 \mu$ ; hyaline conidia ovoid or oblong-elliptical, often  $5-8 \times 3-4 \mu$ , sometimes biguttulate, continuous, at least when first formed; elongate colored conidia 1-6-celled, reaching  $30 \times 3-5 \mu$ , often 1-2-celled,  $12-20 \mu$  long; conidia sometimes greatly enlarged or swollen, swollen conidia at times muriform; germination by hyaline sprout conidia or by germ tubes, often produced apically or subapically but also laterally.

Maculis plerumque brunneus vel fere nigris, usque 3 mm. diam.; fructificationibus conidicis primitus acervulis, demum sporodochiis vel caespitibus plus minusve liberis, in masse obscure olivaceis vel pallide brunneo-olivaceis  $25-70 \mu$  diam.; conidiophoris primitus 1-2-cellulosis, saepe circa  $12 \mu$  altis, ad basim  $2-7 \mu$ , ad apicem attenuatis, acutisve, subobtusatis vel trun-

<sup>1</sup> Jenkins, A. E. The avocado-scab organism. *Phytopath.* 15: 807. 1925.

catis, ex hyphis hyalinis, intraepidermicalibus vel stromatibus prosenchymaticis orientibus, paliformibus, post scissum epidermidis ex conidiis in situ stantibus longioribus, saepe  $25-50\ \mu$ , usque  $100\ \mu$  longis, continuis vel pluriseptatis, rectis vel geniculatis, interdum ramosis; conidiis acrogenis vel pleurogenis, interdum pluribus ex singulo loco orientibus, hyalinis vel coloratis, globosis vel cylindraceis  $2-30 \times 2-5\ \mu$ ; conidiis hyalinis saepe ovoideis vel oblongo-ellipticis,  $5-8 \times 3-4\ \mu$ , plerumque continuis, interdum biguttulatis; conidiis coloratis elongatis, 1-6-cellulosis, usque  $30 \times 3-5\ \mu$ , plerumque 1-2-cellulosis,  $12-20\ \mu$  longis.

Distribution: On avocado, *Persea americana* Mill.; causing the disease known as avocado scab, United States (Florida), Cuba, Puerto Rico, Mexico, South America (Peru), Africa (Union of South Africa and Rhodesia), authority for Peru and Rhodesia based on literature reports.

Type: No. 69844, Mycological Collections, Bureau of Plant Industry; on avocado, Rockdale, Florida, Mar. 21, 1925, W. J. Krome.

Similar to *Sphaceloma fawcettii* Jenkins, but distinguished from it by the generally larger or coarser fructifications, often, in mass, light brownish olive or dark olive.—ANNA E. JENKINS, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.





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## VIROSES OF THE BEAN

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In the course of studies undertaken on the relative resistance of bean varieties to mosaic, it became apparent that the susceptibility of the bean to more than one virus needed further study. The results presented in this paper show that, as in potato, tobacco, and many other plants, several distinct viruses infect bean. This fact has an important bearing on future investigations of any phase of the bean mosaic problem. The present paper reports results of a comparative study of five viruses which infect this plant.

### EARLIER INVESTIGATIONS

The early studies on bean mosaic, begun by Reddick and Stewart (30, 31, 32, 33) in 1917, were concerned with a virus transmissible to many varieties of *Phaseolus vulgaris* L., and one which was carried in the seed. Furthermore it was shown by the same authors that the variety Robust was highly resistant to the disease. In subsequent studies on bean mosaic it seems apparent that Pierce and Hungerford (27), Fajardo (10, 11), and Nelson (26) were concerned with the same virus.

In the meantime a considerable number of other leguminous plants were shown by other investigators (5, 7, 8, 9, 15) to be susceptible to mosaic diseases, but strangely enough none was shown to be identical with the mosaic on bean nor were any of the viruses concerned transmitted to the bean. It was not until Carsner (6) showed that the virus of curly top of sugar beet would produce symptoms on bean that any positive evidence of the susceptibility of the bean to more than one virus was demonstrated. Later, Wingard (35) showed that the tobacco ring-spot virus would infect bean as well as a few other leguminous hosts. Price (28) showed that the ordinary tobacco-mosaic virus would produce local lesions on bean. Nelson (26), on the basis of symptom expression, claimed the existence of another virosis on the bean which he referred to as "rugose mosaic." Zaumeyer

<sup>1</sup> The writer wishes to acknowledge gratefully his indebtedness to Dr. J. C. Walker, under whose direction this work was conducted, for his advice and encouragement throughout the course of the investigation and the preparation of the manuscript; and to Dr. James Johnson who kindly read and criticized the manuscript during its preparation.

(36) has recently reported the transmission of certain legume-mosaic viruses to the bean, but their identity was not established.

For a more extensive review of the literature on the common type of bean mosaic reference may be made to Nelson's (26) recent publication.

#### EXPERIMENTAL MATERIALS AND METHODS

##### Source of Viruses Studied

*Common Bean-Mosaic Virus.* This is the virus of the disease originally described by Stewart and Reddick (33). It is by far the most common and prevalent virus of the bean. For these studies it was secured from mosaic seedlings grown from infected seed. Its correct identity is based on descriptions of the disease by Stewart and Reddick (33) and others, on the properties of the virus as described by Fajardo (11), and on its transmission by seed (32). This virus is designated as *bean virus 1* and is described in detail later in the paper.

*Yellow Bean-Mosaic Virus.* This virus was secured from a plant of the Red Valentine variety, grown in the field at Madison, Wisconsin, in 1931. Its specificity was first suspected not only from the fact that it produced symptoms distinct from that of the common bean-mosaic virus, but also because it affected the varieties, Corbett Refugee, Great Northern UI No. 1, and Robust, which are resistant to common mosaic. This virus is designated as *bean virus 2* and also is described in detail later.

*Alfalfa-Mosaic Virus.* This virus was secured from a strain of Turkistan alfalfa grown by Dr. F. R. Jones. The affected plants had been removed from the field and potted in the greenhouse in the autumn of 1931. The plants were decidedly dwarfed and the leaves distinctly mottled. The extracted juice from such plants, when inoculated to bean, produced necrotic lesions on certain varieties.

This disease on alfalfa appeared to be distinct from the mosaic on the same species described by Weimer (34). With his permission the virus he described in designated as *alfalfa virus 1*, while that from alfalfa used by the writer is herein referred to as *alfalfa virus 2*.

*Ordinary Tobacco-Mosaic Virus.* This virus, described by Johnson (21) as *tobacco virus 1*, has been shown by Price (28) to produce local necrotic lesions on bean. Inoculum for these studies was secured by the writer from Dr. James Johnson.

*Tobacco Ring-Spot Virus.* Ring-spot of tobacco was first described by Fromme and Wingard (13). The properties and host range of the virus were discussed in later papers from the same laboratory (14, 29, 35). Wingard (35) reported its successful transmission to bean. It was secured by the writer from Johnson and Grant (22), who in turn had received it from Wingard.

### Source of Bean Varieties

Twenty-four varieties of common bean were used, all of which are listed in Table 1. Many of these were furnished by Associated Seed Growers Inc., New Haven, Connecticut. Corbett Refugee is a selection, resistant to common bean mosaic, derived from a single plant of Refugee Green<sup>2</sup> variety by Ralph Corbett of the Sioux City Seed Company, Sioux City, Iowa, who supplied seed to the writer. Great Northern UI No. 1 was selected for mosaic resistance from commercial Great Northern by the writer at the Idaho Agricultural Experiment Station, where seed of both these strains was secured for this work. Robust and Mexican Tree varieties were supplied by the Michigan Agricultural Experiment Station. Seed of Refugee Green variety was furnished by E. J. Renard of the Cannery Seed Corporation, Beaver Dam, Wisconsin. Wisconsin Hybrid Wax No. 536 was secured from G. H. Rieman, formerly of the United States Department of Agriculture. Seeds of other leguminous plants were secured from various sources and, as far as could be determined, were true to name.

### Methods

The studies were carried out partly in the greenhouse, where the temperature was usually held at 24–28° C., and in field plots on the experiment station farm at Madison, Wisconsin. Inoculum was prepared by crushing leaves and stems of infected plants in a sterile mortar and straining through cheesecloth. Bean plants were inoculated in the young two-leaf stage by rubbing the upper surfaces of the first leaves with a small piece of cheesecloth that had been immersed in the inoculum. Other host plants were inoculated by the same method. Where symptoms on inoculated plants were very mild or completely masked, the presence or absence of the virus was determined in some instances by extracting the juice and inoculating to healthy Refugee Green plants grown from a mosaic-free stock of seed, and used for this purpose because of its high susceptibility to all the viruses studied.

The properties of the viruses were determined on greenhouse-grown plants by the same methods described by Johnson and Grant (22).

In the experiments on aphid transmission the method described by Hoggan (18) was used.

### EXPERIMENTAL RESULTS

The comparative studies of the five viruses under consideration were first based upon symptom expression and varietal susceptibility on 24 varieties of the common bean. Further points of differentiation are to be found

<sup>2</sup> This variety is known in the seed trade as Refugee Green, Stringless Green Refugee, and Late Stringless Refugee Green Pod.

in the results presented on the properties of the viruses *in vitro* and on their modes of transmission. The susceptibility or resistance of a number of other leguminous species also was determined. It will be seen that host reaction becomes an important criterion of distinction between the viruses.

### Symptom Expression

*Common Bean Mosaic (bean virus 1).*—On account of the known variation in susceptibility of bean varieties to *bean virus 1*, the study of symptom expression on the 24 varieties was simplified by dividing them into 3 classes according to their resistance to *bean virus 1*. Class I consists of susceptible varieties on which pronounced symptoms are produced. Class II may be defined as the tolerant class. These varieties are infected as readily by inoculation as those in Class I but do not ordinarily develop marked symptoms. However, the virus may be recovered readily from inoculated plants even when the disease is completely masked. Class III comprises those varieties that are immune from *bean virus 1*. They develop no symptoms on inoculation and the virus cannot be recovered from inoculated plants. In table 1 the 24 varieties are listed in their respective classes. The symptoms of common bean mosaic (*bean virus 1*) have been described by Stewart and Reddick (33), Barss (4), Pierce and Hungerford (27), Fajardo (10), and Nelson (26); but, since both *bean virus 1* and *bean virus 2* cause systemic infection with some overlapping of symptoms, the signs of both will be described on Refugee Green and Mexican Tree from Class I, Giant Stringless and Brittle Wax from Class II, and Corbett Refugee, Robust, and Great Northern UI No. 1 from Class III.

When the primary leaves of the susceptible Refugee Green are inoculated with *bean virus 1*, symptoms first appear as a slight chlorosis of the first trifoliate leaf, which tends to curl but, ordinarily, shows no mottling. The leaflets of the second trifoliate leaf become somewhat elongated, with the lamina curled downward. Mottling occurs in various patterns, but, commonly, the background is of a somewhat lighter green than normal, with dark green areas interspersed on the light green field. The dark green often appears as a band along the midvein (Fig. 1, D). Under greenhouse conditions, during the winter months, the mottling may be almost entirely masked and only a slight curling or a faint chlorosis gives evidence of the presence of the virus. Under field conditions mottling may be accentuated to the extent that the dark green areas become savoyed (Fig. 1, E). The downward curling is a rather constant feature and is one of the significant ways in which it differs from yellow bean mosaic. The sequence of symptoms, as growth continues, may show considerable variation, but the stunting, curling, and mottling so evident on diseased plants during their first 2 months of growth, usually become less pronounced as the plants approach

TABLE 1.—*Comparison of the susceptibility of certain bean varieties to common bean mosaic (bean virus 1)*

Class	Variety	Inoculated in the greenhouse		Inoculated in the field, 1931		Natural infection in the field, 1932	
		Inoculated	Infected	Inoculated	Infected	Plants	Infected
		Number	Per cent	Number	Per cent	Number	Per cent
I. Susceptible	Dwarf Horticultural	35	90	37	97	124	95
	Comm. Great Northern	51	82	32	79	123	86
	Mexican Tree	53	85	37	94	176	86
	Red Kidney	40	92	20	85	118	99
	Red Valentine	46	91	43	100	104	95
	Refugee Green	180	90	57	96	784	93
	Refugee Wax	40	92	53	94	123	100
II. Tolerant	Well's Red Kidney	31	84			28	75
	Black Valentine	40	77	40	95	105	89
	Bountiful	40	85	33	93	122	87
	Brittle Wax	46	82	57	84	125	75
	Burpee's S. G. Pod	42	83	36	94	129	79
	Davis Wax	35	91	36	83	101	76
	Full Measure	36	80	34	93	119	83
III. Immune	Giant S. G. Pod	49	80	68	91	115	89
	Imp. Kidney Wax	40	75	64	78	148	73
	Longfellow	40	70	46	79	114	82
	Pencil Pod	40	77	44	86	156	83
	Sure Crop Wax	40	85	42	89	121	96
	Unrivalled Wax	52	8			68	4
	Corbett Refugee	192	0 <sup>a</sup>	56	0	1110	1 + <sup>b</sup>
	Great Northern UI No. 1	90	0 <sup>a</sup>	100	2 <sup>b</sup>	117	3 - <sup>b</sup>
	Robust	119	0 <sup>a</sup>	154	4 <sup>b</sup>	87	2 + <sup>b</sup>
	Wis. Hybrid Wax No. 536	70	2 -	112	9 <sup>b</sup>	57	3 + <sup>b</sup>

<sup>a</sup> Transfer inoculations to Refugee Green demonstrated that bean virus 1 was not present.<sup>b</sup> Test inoculations in the greenhouse showed the mosaic infection on these varieties to be due to bean virus 2 and not to bean virus 1.

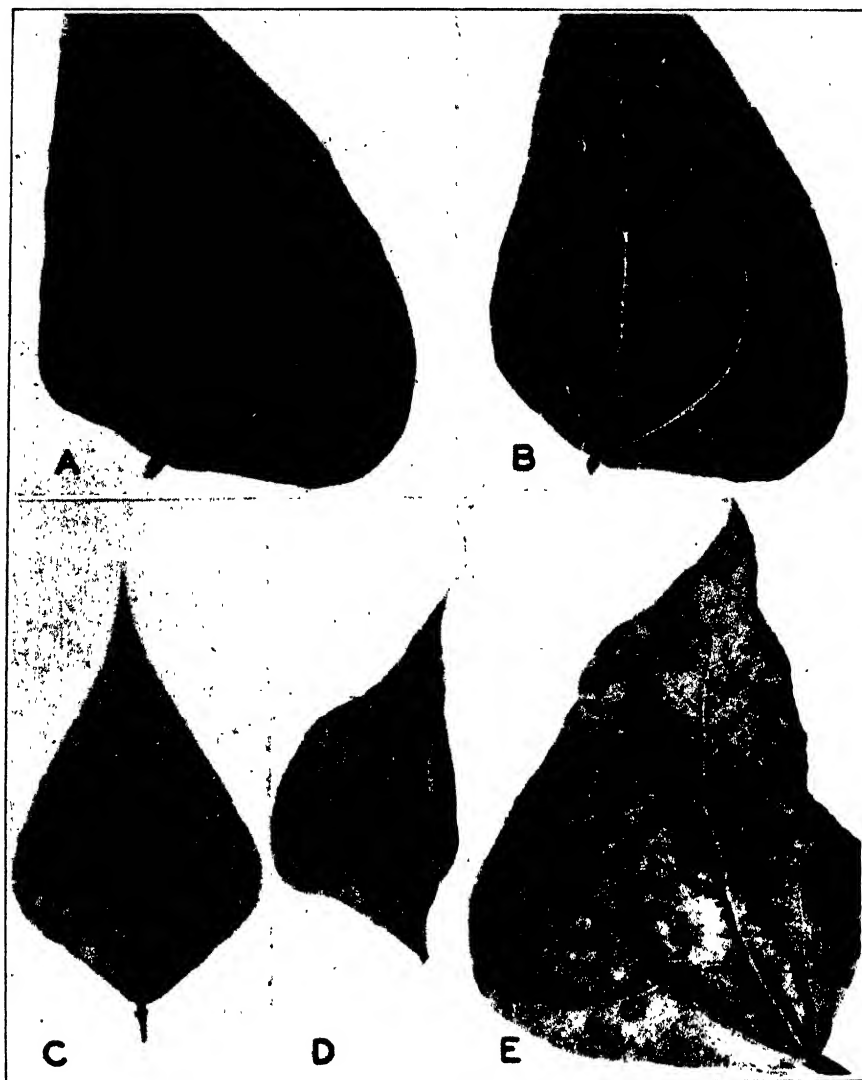


FIG. 1. Variation in leaf symptoms exhibited by different varieties of bean inoculated with *bean virus 1*. A. Corbett Refugee noninoculated control. B. Corbett Refugee inoculated but showing no symptoms. C. Giant Stringless, showing no symptom other than reduction in size. D. Refugee Green showing chlorosis with dark green area along midvein. E. Refugee Green with severe symptoms.

maturity. While yield is reduced and maturity delayed, the effects are not nearly so pronounced with *bean virus 1* as with *bean virus 2*.

On the Mexican Tree variety the symptoms are much like those described for Refugee Green, except that in the second trifoliate leaf the dark green

areas more commonly follow along the midribs and extend along the lateral veins towards the margin of the leaflets, whereas the intervenal areas are light green in color.

On Giant Stringless and Brittle Wax varieties the symptoms are very mild under greenhouse conditions (Fig. 1, C). In some cases dark green areas can be detected along the veins of the second trifoliates of plants inoculated in the primary leaf stage. In the subsequently developing leaves it is often impossible to detect mosaic symptoms. To determine infection on these varieties it is often necessary to make transfer inoculations to a more susceptible variety, such as Refugee Green.

Corbett Refugee, Robust, and Great Northern UI No. 1 are apparently immune from *bean virus 1*. Repeated inoculations failed to produce symptoms (Fig. 1, A, B). Juice extracted from inoculated plants of the last-named varieties fails to produce symptoms on the Refugee Green variety.

*Yellow Bean Mosaic (bean virus 2).*—On Refugee Green, *bean virus 2* is much more virulent than the former virus (Fig. 2, A–C). Inoculation



FIG. 2. Refugee Green plants six weeks after inoculation. A. Control inoculated with water. B. Inoculated with *bean virus 1*. The leaves are mottled but there is little reduction in size of plants. C. Inoculated with *bean virus 2*. Note extreme reduction in size of the plant.

on the primary leaves usually produces a distinctive drooping of the developing trifoliate leaf. Each leaflet is not only curled downward but, in addition, is definitely pointed downward from the point of attachment to the petiole. This is a very simple method of distinguishing between *bean virus 1* and *bean virus 2*. The surfaces of the leaflets are slightly irregular, and small, light yellow spots soon develop in the dark green background of the first trifoliate. The yellowing gradually spreads over the entire surface, causing the leaflets to become more or less chlorotic. In these early stages the young growth has a tendency to become brittle. The first trifoliate leaflets do not remain curled downward, but, as they enlarge, become slightly concave on their upper surfaces and take on a glossy appearance.

On the third and fourth trifoliate leaves there is a very distinct mottling of yellow green and dark green areas, which stand out in greater contrast to each other than is the case with common bean mosaic (Fig. 3, F). The

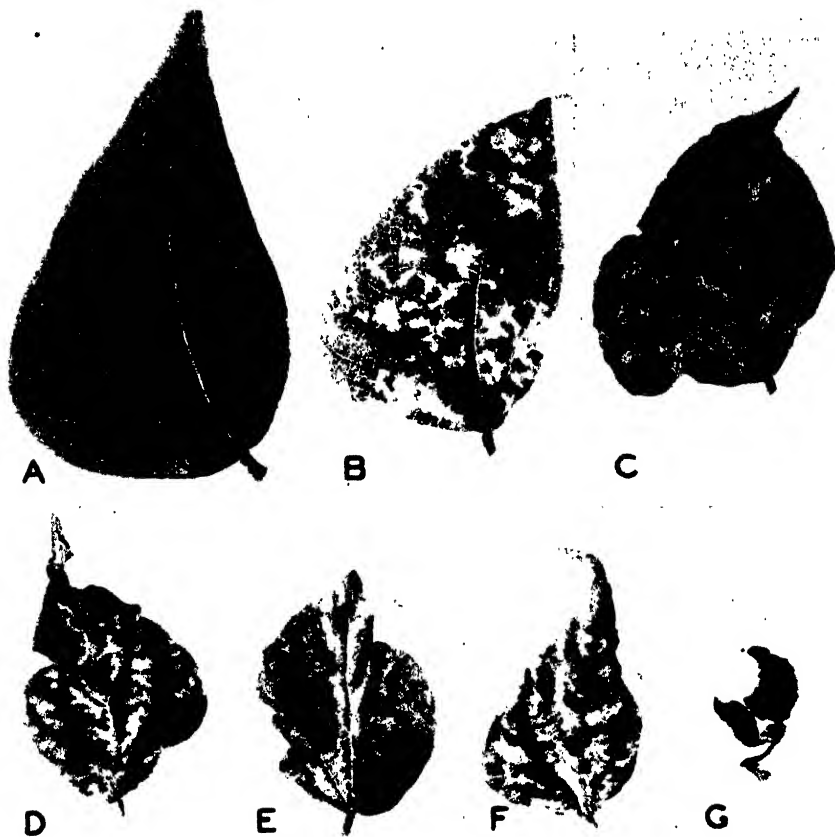


FIG. 3. Variation in leaf symptoms exhibited by different varieties of bean infected with *bean virus 2*. A. Corbett Refugee healthy. B. Corbett Refugee showing mottling. C. Robust showing mottling. D. Brittle Wax with mottling and malformation. E. Giant Stringless with mottling and malformation. F. Refugee Green showing mottling and reduced size. G. Mexican Tree with complete killing of leaflets.

downward curling usually associated with the latter is not a constant characteristic of yellow bean mosaic in the later stages of development. In plants affected with yellow bean mosaic, symptoms do not become masked in their later stages of growth, but the mottling becomes more pronounced as the season progresses. Plants become decidedly stunted and bushy due to a reduction in the length of the internodes and a proliferation of branches. Maturity is greatly delayed and production of pods is greatly reduced.



When the Mexican Tree variety is inoculated on the primary leaves a lethal effect upon the developing trifoliate leaves is produced (Fig. 3, G). Usually the young epicotyl is entirely killed. In some cases growth continues from the buds in the axils of the primary leaves, but the plants never develop sufficiently to blossom and set fruit. The secondary growth is decidedly proliferated, mottled, and malformed.

The leaves of Giant Stringless and Brittle Wax have a tendency to curl and become otherwise malformed. The lighter-colored portions of the mottling are in some cases devoid of all green color (Fig. 3, D, E). These light-colored areas are usually depressed somewhat from the convex surface of the curled leaf. Stunting and proliferation are not so marked as on Mexican Tree. The pods of affected plants become distorted and mottled.

The first symptoms following inoculation on Corbett Refugee, Robust, and Great Northern UI No. 1 are similar to those described on Refugee Green. In the later stages of development, however, the plants are not so severely dwarfed and, even though mottling is often pronounced, the set of pods is not appreciably reduced (Fig. 3, A-C). There is no curling of the leaves.

*Alfalfa Virus 2.* This virus produces local necrotic lesions on Refugee Green beans within 2 days following inoculation. The lesions appear first as minute light brown spots about  $\frac{1}{2}$  mm. wide. They gradually widen and attain a maximum diameter of 1 to 2 mm. in 6 to 8 days. The color of fully formed lesions is chocolate brown. The lesions formed on Red Valentine and certain other varieties are slightly larger than those on Refugee Green (Fig. 4, A). The disease does not become systemic on any of the varieties of *Phaseolus vulgaris* tested.

*Tobacco Virus 1.* This virus also produces local necrotic lesions on Refugee Green beans. Price (28) described the lesions caused by *tobacco virus 1* as from less than  $\frac{1}{2}$  to 1 mm. wide and consisting of pale necrotic areas surrounded by a ring of dark red tissue. The writer obtained similar results. The lesions develop more slowly and do not become so large as those caused by *alfalfa virus 2* (Fig. 5, C). No systemic effect on the plants was observed.

*Tobacco Ring-spot Virus.* The symptoms of tobacco ring spot on bean also vary somewhat with different varieties. The symptoms on Robust and Mexican Tree consist of local lesions that are necrotic throughout (Fig. 5, B). These lesions gradually extend until they coalesce and the leaf collapses. On other varieties they are much less distinct, appearing as necrotic rings in the normal tissue or as a vein necrosis (Fig. 4, B). Following the development of local lesions on the inoculated leaves, symptoms appear in a few days on the young noninoculated leaves. This systemic infection causes the death of the entire plant. Corbett Refugee, Robust,

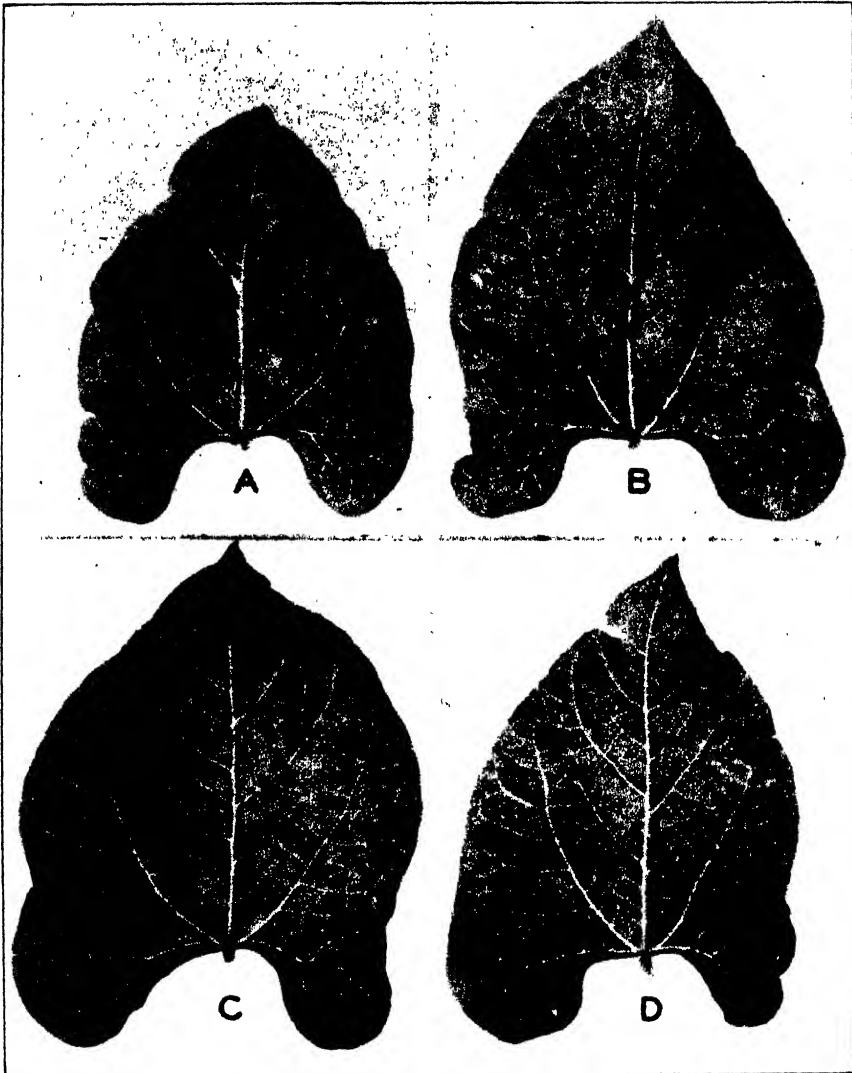


FIG. 4. Primary leaves of Red Valentine bean showing various symptoms. A. Necrotic local lesions caused by inoculation with *alfalfa virus 2*. B. Local lesions and vein necrosis caused by tobacco ring-spot virus. C. No local lesions from inoculation with *tobacco virus 1*. D. Noninoculated control.

and Mexican Tree, however, seldom become systemically infected with ring spot.

#### Resistance and Susceptibility in Bean Varieties

*Common Bean Mosaic* (*bean virus 1*). All 24 varieties were inoculated in the greenhouse and further tests were made in the field. In the green-

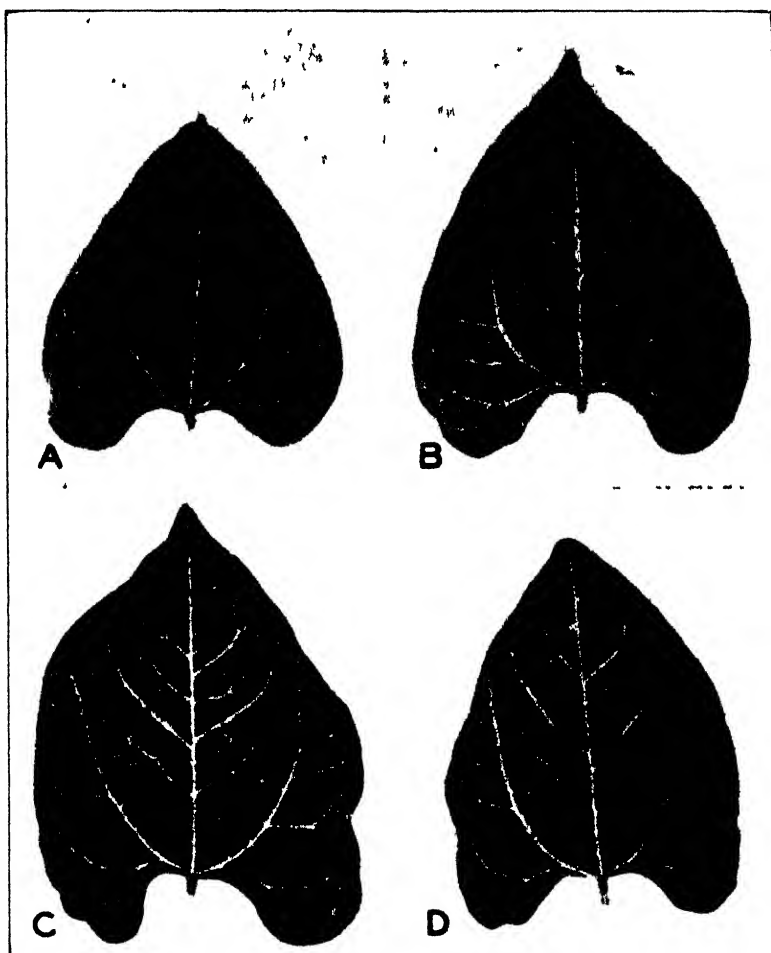


FIG. 5. Primary leaves of Mexican Tree bean showing necrotic local lesions produced by inoculations with different viruses. A *Alfalfa virus 2*. Only a few lesions. B *Tobacco ring spot virus*. C *Tobacco virus 1*. D. Noninoculated control.

house each plant was inoculated twice, once on the primary leaves, and once on the first trifoliate leaf. In the summary of results given in table 1, the varieties are divided into the 3 classes discussed under symptoms. These will be referred to for convenience as Class I, susceptible; Class II, tolerant; and Class III, immune. They are probably similar to those classes designated by Reddick and Stewart (30, 31) who divided a much longer list of varieties into "highly susceptible," "intermediate," "resistant," and "immune" classes.

The percentage of plants that showed symptoms in Classes I and II is generally high, with the exception of Unrivalled Wax. The distinction be-

tween these groups, however, is not one of percentage of infected plants but that of tolerance and disease expression. Plants in Class I usually exhibited marked symptoms. In Class II, plants that became diseased showed only mild symptoms. Some of the plants that showed slight symptoms and some that showed no symptoms were selected from inoculated populations of Brittle Wax and Giant Stringless varieties. When the juice from these was inoculated into healthy Refugee Green plants, a high percentage of the plants were shown to have had the virus present, regardless of whether or not they showed visible symptoms of mosaic.

In Class III, Robust, Corbett Refugee, and Great Northern UI No. 1 plants showed no mosaic symptoms whatever (Fig. 1, A, B). Juice was extracted from each of 68 inoculated Corbett Refugee plants, 25 of Great Northern UI No. 1, and 30 of Robust and transferred to healthy Refugee Green plants. No signs of mosaic resulted from any of these inoculations. Wisconsin Hybrid Wax showed symptoms on one of the 70 plants used. Juice extracted from 17 plants in this variety when inoculated to healthy Refugee Green plants failed to produce the disease in any case except that of juice extracted from the one plant that had shown symptoms. This exception is not clear but since it occurred in only one plant, which might have been the result of seed admixture or even a field cross, the variety is placed tentatively in Class III.

The field inoculations in 1931 and exposure to natural infection in 1932 confirmed the results from the greenhouse. Varieties in Class III all remained free from common bean mosaic, although a small percentage showed natural infection with yellow bean mosaic. Each of these plants was tested by transferring juice to healthy Refugee Green and was shown to be affected with only yellow bean mosaic. Of Class II all showed a high percentage of mild infection except Unrivalled Wax. Three plants of this variety out of 68 became infected with common bean mosaic and 2 with yellow bean mosaic. In Class I all varieties were severely diseased.

*Yellow Bean Mosaic (bean virus 2).* Greenhouse inoculations showed that all varieties were susceptible to yellow bean mosaic (Table 2). However, the data lend support to the classification scheme set up with respect to varietal susceptibility to *bean virus 1*. The immune varieties of Class III, though somewhat dwarfed at first, and later characterized by a distinct mottling of the leaves, usually developed into full-size plants. Serious injury in the field was not observed and the natural occurrence of *bean virus 2* on varieties of Class III was limited to a small percentage of plants. In a Corbett Refugee planting of 1110 plants in 1932 only 13 were affected with yellow bean mosaic. Only small percentages of infected plants occurred in the Great Northern UI No. 1 and Robust varieties grown at Madison, Wisconsin, in 1931 and 1932. Yellow bean mosaic in the field is par-

TABLE 2.—*Relative susceptibility of varieties of bean to bean virus 2, alfalfa virus 2, tobacco virus 1, and tobacco ring-spot virus based on greenhouse inoculations*

Variety	Bean virus 2 <sup>a</sup>			Alfalfa virus 2 <sup>b</sup>			Tobacco virus 1 <sup>b</sup>			Ring-spot virus		
	Total plants inoculated	Per cent systemically infected	Total plants inoculated	Per cent locally infected	Ave. no. lesions per leaf	Total plants inoculated	Per cent locally infected	Ave. no. lesions per leaf	Total plants inoculated	Per cent systemically infected	Per cent locally infected	Ave. no. lesions per leaf
Dwarf Horticultural	23	60	26	100	16	31	0	0	16	100	50	2
Comm. Great Northern	41	27	26	50	1	24	100	35	19	52	30	1
Mexican Tree	38	90	28	100	2	29	100	58	17	17	66	1
Red Kidney	23	56	19	100	35	23	0	0	5	100	100	5
Red Valentine	27	96	28	100	50	28	0	0	17	100	100	20
Refugee Green	52	75	28	100	34	27	100	30	17	100	100	2
Refugee Wax	38	52	26	100	26	28	100	30	16	100	100	18
Well's Red Kidney	30	50	21	100	44	27	0	0	16	100	68	4
Black Valentine	39	66	26	100	32	32	0	0	19	100	100	13
Bountiful	20	60	24	100	23	30	0	0	16	100	100	30
Brittle Wax	42	78	21	100	50	21	0	0	13	100	100	20
Burpee's S. G. Pod	23	56	24	100	40	29	0	0	16	100	100	20
Davis Wax	31	42	12	100	32	16	0	0	6	100	100	13
Full Measure	20	95	26	100	40	27	0	0	18	100	100	15
Giant S. G. Pod	47	47	27	100	33	28	0	0	15	100	100	14
Imp. Kidney Wax	24	62	19	100	44	18	0	0	11	100	100	16
Longfellow	26	59	26	100	15	23	0	0	14	100	100	3
Pencil Pod	31	45	24	100	36	27	0	0	16	100	100	22
Sure Crop Wax	28	57	28	100	48	29	0	0	11	100	100	15
Unvalled Wax	47	12	27	100	45	30	0	0	18	100	100	22
Corbett Refugee	48	50	26	20	1	28	100	20	18	0	100	3
Great Northern U1 No. 1	39	33	40	50	1	21	90	23	16	94	50	1
Robust	42	60	34	50	1	31	100	35	17	12	100	6
Wis. Hybrid Wax No. 536	44	14	28	100	46	26	19	1	15	100	50	1

<sup>a</sup> No local necrosis produced by bean virus 1 nor by bean virus 2.<sup>b</sup> No systemic infection produced by alfalfa virus 2 nor by tobacco virus 1.

ticularly noticeable on these varieties, since there is no confusion with common bean mosaic. It is more difficult to distinguish accurately between the two diseases on a variety like Refugee Green, which is susceptible to both. Most varieties in Class I were very severely stunted. In some cases they were killed outright as described earlier under symptoms on Mexican Tree variety. Red Kidney and Well's Red Kidney were exceptional in that they were more tolerant to yellow bean mosaic than most varieties in Classes II and III. In general, the varieties of Class II were less severely affected than were those of Class I.

*Alfalfa Virus 2*.—The data in table 2 indicate the percentage of plants infected and the average number of local necrotic lesions per leaf. All varieties showed some lesions. Of those highly resistant to *bean virus 1* and *bean virus 2*, Corbett Refugee, Great Northern UI No. 1, and Robust showed very few lesions on 50 per cent or less of the plants. Wisconsin Hybrid Wax showed no marked resistance. Of those varieties very susceptible to *bean viruses 1* and *2*, Great Northern and Mexican Tree were highly resistant to *alfalfa virus 2* (Fig. 5, A). Thus resistance to this virus is not correlated with that to the two bean-mosaic viruses.

*Alfalfa Virus 1*.—Price (28) tested a large number of bean varieties and found many that did not develop lesions when inoculated with this virus. Many of the varieties considered here (Table 2) showed complete resistance (Fig. 4, C). There is no correlation, however, between resistance to this virus with that to the two bean viruses. Many of the varieties susceptible to *tobacco virus 1* showed some resistance to *alfalfa virus 2* (Fig. 5, A, C), but here, again, the correlation is not complete.

*Tobacco Ring-spot Virus*.—As shown in table 2, all varieties are affected, usually with local necrotic lesions on the inoculated leaf and systemic necrotic symptoms on the younger portions of the plant. Corbett Refugee was the only one that showed no systemic infection, but a few local lesions were produced. Of the varieties Robust, Mexican Tree, and Great Northern only 12 to 52 per cent of the plants were systemically infected, and thus appeared to have some tolerance. All other varieties were severely diseased.

#### PROPERTIES

The properties of common bean mosaic were studied by Fajardo (11). His work has been repeated in part for the purpose of obtaining a direct comparison of the properties of *bean virus 1* with those of *bean virus 2*, *alfalfa virus 2*, and the tobacco ring-spot virus. The properties of *tobacco virus 1* were not studied, for they have received attention by Allard (1, 2, 3) and many others. The property tests on all the viruses were made in the same manner and under as uniform conditions as possible. In these tests five bean plants were used as a test unit. In the summary in tables 3

TABLE 3.—Comparison of certain properties of bean virus 1, bean virus 2, and tobacco ring-sp<sup>a</sup> virus, as determined by systemic infection on Refugee Green bean

Temp. °C.	Thermal death point			Longevity in vitro				Tolerance to dilution				Resistance to chemicals <sup>a</sup>			
	Bean virus 1	Bean virus 2	Tobacco ring-sp <sup>a</sup> virus	Time aged	Bean virus 1	Bean virus 2	Tobacco ring-sp <sup>a</sup> virus	Dilution	Bean virus 1	Bean virus 2	Tobacco ring-sp <sup>a</sup> virus	Chemical	Strength of chemical	Bean virus 1	Bean virus 2
Inoc. Control	25 <sup>b</sup> 19	25 15	10 10	None	25 20	25 21	10 10	None	25 17	25 17	10 10	None		25 21	25 16
54	25 16	25 8		20 hours	25 15	25 9		1-10	25 20	25 17	10 10	Alcohol	25%	25 10	25 9
56	25 10	25 4	10 10	24 do	25 7	25 5	10 10	1-100	25 10	25 15	10 10	do	50%	25 0	25 0
58	25 0	25		28 do	25 2	25		1-200	25 8	25 11		do	75%		
62			10 10	32 do	25 0	25 0		1-400	25 5	25 9		Nitric acid	1-500	25 16	25 13
64			10 7	3 days			10 10	1-800	25 3	25 3		do	1-200	25 0	25 0
66			10 0	5 do			10 5	1-1000	25 2	25 0	10 3	do	1-100		
				7 do			10 4	1-2000	25 0	25 0	10 0	Formaldehyde	1-2000	25 13	25 8
				8 do			10 1					do	1-1000	25 7	25 0
				9 do			10 0					do	1-500	25 0	25 0
												do	1-200		
												do	1-100		

<sup>a</sup> 30-minute treatments.  
<sup>b</sup> Upper figure is number of plants inoculated; lower figure, number of plants infected.  
<sup>c</sup> Concentrated solution, which contains approximately 37 per cent formaldehyde.

and 4 the results of 5 tests for each property determined have been totalled. It should be pointed out that minor variations occurred in the individual trials that are not always apparent in the summarized results. The properties of *alfalfa virus 2* were tested by its ability to produce local lesions on Refugee Green bean (Table 4). One-half of the tobacco ring-spot virus determinations were based upon local infection on Henderson Bush Lima (Table 4), and one-half on systemic infection on Refugee Green bean (Table 3). In some of the trials with ring spot, Refugee Green beans appeared to be slightly more sensitive to systemic infection than was Henderson Bush Lima bean to local infection.

*Thermal Inactivation Point.* (Tables 3 and 4). The thermal inactivation point of *bean virus 1* was found to approximate the upper limits set by Fajardo (11). *Bean viruses 1* and *2* were both inactivated at 56° to 58° C. when exposed at this temperature for 10 minutes. (Table 3). *Alfalfa virus 2* was inactivated at 62° to 64° C. (Table 4). Inactivation of the tobacco ring-spot virus at 66° C. is in general agreement with the determinations by Henderson and Wingard (17) and Johnson and Grant (22).

The thermal death point of *tobacco virus 1*, being generally accepted at about 90° C., it is apparent that, with the exception of the close similarity between the 2 bean viruses, the 5 viruses considered in this paper have rather distinct thermal properties.

*Resistance to Aging in Vitro.* (Tables 3 and 4). *Bean viruses 1* and *2*, in all trials, lost their infectivity after aging *in vitro* 24 to 32 hours. Fajardo (11) had previously shown *bean virus 1* to be inactivated at 24 hours. *Alfalfa virus 2* and the tobacco ring-spot virus were both inactivated after 7 to 9 days. Priode (29) found the ring-spot virus to be still infective after aging 7 days at 10° C. Johnson and Grant (22) found it to be inactivated in 5 to 6 days at room temperature. All the above viruses afford a striking contrast to *tobacco virus 1*, which is known to remain infective *in vitro* for two years or more (2, 21).

*Tolerance to Dilution.* (Tables 3 and 4). Dilution tests showed no significant differences between *bean virus 1*, *bean virus 2*, *alfalfa virus 2*, and the tobacco ring-spot virus. All lost their infectivity at dilutions greater than 1 to 1000. Infection with *bean virus 2* was not obtained with dilutions greater than 1 to 800, but this cannot be considered a significant difference from the 1 to 1000 dilution noted in this study and also by Fajardo (11) for *bean virus 1*. No infection was obtained with the tobacco ring-spot virus at dilutions greater than 1 to 1000. Henderson and Wingard (17), however, obtained a trace of infection at 1-10,000. All the above viruses are distinct from *tobacco virus 1*, which has been shown by Allard (1) and by Johnson and Grant (22) to be infective at a dilution of 1 to 1,000,000.



TABLE 4.—Comparison of certain properties of alfalfa virus 2 and tobacco ring-spot virus, as determined by the production of local lesions on bean

Temp. °C.	Thermal death point		Longevity <i>in vitro</i>			Tolerance to dilution			Resistance to chemicals <sup>a</sup>			Tobacco ring- spot virus
	Alfalfa virus 2	Tobacco ring- spot virus	Time aged days	Alfalfa virus 2	Tobacco ring- spot virus	Dilution	Alfalfa virus 2	Tobacco ring- spot virus	Chemical	Strength of chemical	Alfalfa virus 2	
Inoc.	2102 <sup>b</sup>	677 <sup>c</sup>	None	4185 <sup>b</sup>	198 <sup>c</sup>	None	3904 <sup>b</sup>	300 <sup>c</sup>	None		4919 <sup>b</sup>	277 <sup>c</sup>
58	1701		1	3051	109	1-10	1952	38	Alcohol	25%	980	103
60	1084		3	855	31	1-100	368	14	do	50%	50	41
62	379	34	5	408	6	1-200	180		do	75%	0	0
64	0	5	7	62	0	1-400	89		Nitric	1-500	25	145
66		0	8	5	0	1-800	31	0	acid	1-200	0	19
			9	0	0	1-1000	13	0	do	1-100		0
						1-2000	1	0	Formalde- hyde	1-1000		161
									do	1-500	1675	36
									do	1-200	83	0
									do	1-100	0	0

<sup>a</sup> 30-minute treatments.  
<sup>b</sup> Figures are total lesions produced on 50 inoculated primary leaves of Refugee Green bean.  
<sup>c</sup> Figures are total lesions produced on 20 inoculated primary leaves of Henderson Bush Lima.

*Resistance to Chemicals.* (Tables 3 and 4). Allard (2), Johnson (21), and Johnson and Grant (22) have shown that *tobacco virus 1* has a high resistance to various chemical treatments. Fajardo (11) obtained inactivation of *bean virus 1* with 25 per cent alcohol for 30 minutes. In the present investigation *bean virus 1* withstood treatment with 25 per cent alcohol, but was always inactivated by 50 per cent alcohol. *Bean virus 2* also was inactivated by 50 per cent alcohol and *alfalfa virus 2* was inactivated by 75 per cent alcohol. The tobacco ring-spot virus was not inactivated by 75 per cent alcohol when the inoculation tests were made on Refugee Green beans, but the tests on Henderson Bush Lima gave no local lesions after treatment with this strength of alcohol. The tobacco ring-spot virus withstood a 30-minute treatment with nitric acid, 1-200, but *bean viruses 1* and *2* and *alfalfa virus 2* were all inactivated at this concentration. In the trials of Johnson and Grant (22) *tobacco virus 1* was not inactivated by nitric acid, 1-200, for 48 hours.

The infectivity of the viruses in question was destroyed by 30-minute treatments with 37 per cent formaldehyde at concentrations as follows: *bean virus 1* at 1-500; *bean virus 2* at 1-1000; *alfalfa virus 2* and tobacco ring-spot virus at 1-100. The tobacco ring-spot virus, however, did not produce local lesions on Henderson Bush Lima after treatment with 1-200 formaldehyde.

#### Transmission

*By Insects.* Transmission of bean mosaic (*bean virus 1*) by aphids was accomplished by Nelson (25) with the potato aphid, *Macrosiphum solanifolii* Ashm. Fajardo (10) reported successful transmission with *Aphis rumicis* L. and *Myzus persicae* Sulz. Zaumeyer (37), in addition to the above, obtained transmission with *Illinoia pisi* Kalt., *Aphis gossypii*, *Brevicoryne brassicae*, *Hyalopecterus atriplicis*, and *Macrosiphum ambrosia*.

The writer secured transmission of *bean viruses 1* and *2* with both the pea aphid *Illinoia pisi* and the potato aphid, *Macrosiphum solanifolii*, thus confirming the previous reports of aphid transmission of *bean virus 1*. Systemic infection of *alfalfa virus 2* on red clover, white clover, and Perfection peas, and local infection on Refugee Green beans was obtained with the pea aphid, *Illinoia pisi*.

No aphid transmission tests were made with the tobacco ring-spot virus nor with *tobacco virus 1*. Hoggan (18, 19) has shown that *tobacco virus 1* is not transmitted by the peach aphid *Myzus persicae* and that *Myzus pseudosolani*, *Macrosiphum solanifolii*, and *Myzus circumflexus* are able to transmit *tobacco virus 1* from tomato to tobacco but not from tobacco to tobacco. Fenne (12) obtained negative results in attempts to transmit tobacco ring-spot virus by means of tobacco flea beetle, cucumber flea beetle, leaf hopper, aphid, firefly, and tobacco hornworm.

*Through Seed.* That the virus of common bean mosaic (*bean virus 1*) is transmitted through the seed of the common bean was first proved by Reddick and Stewart (32). Subsequent investigations by Pierce and Hungerford (27), Fajardo (10), and Nelson (26) have shown that, when large populations of seed from diseased plants are tested, from 30 to 50 per cent diseased seedlings are produced. Similar percentages were obtained in the present study.

In a total of 6532 seedlings grown from seed of plants infected with *bean virus 2* none were found to be affected with yellow bean mosaic (Table 5). These seedlings were grown from seed of varieties susceptible to both *bean virus 2* and *bean virus 1* and of varieties susceptible only to *bean virus 2*. They included seed from naturally infected plants from different

TABLE 5.—Results of tests on seed transmission of *bean virus 2*

Variety	History of infected plants from which seed was obtained	Number of seedlings grown	Number infected with <i>bean virus 2</i>
Corbett Refugee	Naturally infected in field. Billings, Montana. 1931.	340	0
	Naturally infected in field. Madison, Wisconsin. 1932.	2469	0
	Artificially infected in field. Madison, Wisconsin. 1932.	64	0
	Artificially infected in greenhouse. Madison, Wisconsin. 1931-32.	140	0
Great Northern UI No. 1	Naturally infected in field. Twin Falls, Idaho. 1931.	576	0
	Naturally infected in field. Madison, Wisconsin. 1931.	1171	0
	Artificially infected in greenhouse. Madison, Wisconsin. 1931.	15	0
Robust	Naturally infected in field. Madison, Wisconsin. 1931.	1023	0
	Artificially infected in field. Madison, Wisconsin. 1932.	73	0
	Artificially infected in greenhouse. Madison, Wisconsin. 1931-32.	72	0
Wis. Hybrid Wax No. 536	Artificially infected in field. Madison, Wisconsin. 1932.	31	0
Varieties susceptible to <i>bean virus 1</i>	Artificially infected in field. Madison, Wisconsin. 1932. Naturally infected with <i>bean virus 1</i> .	506	0
Manchu soybean	Artificially infected in greenhouse. Madison, Wisconsin. 1932.	52	0
Total .....		6532	0

sources, as well as seed from plants artificially infected in the greenhouse with *bean virus 2*.

It is of interest to note the outcome of tests made with seeds obtained from plants infected with both *bean virus 1* and *bean virus 2*. A total of 506 seedlings (Table 5) were grown from seed harvested from field-grown plants that had been artificially infected with *bean virus 2* and naturally infected with *bean virus 1*. No evidence of the transmission of *bean virus 2* was obtained. However, a small percentage of the 506 seedlings exhibited symptoms of common bean mosaic; and transfer inoculations established conclusively that these mosaic plants were caused by the virus of common bean mosaic alone. The nontransmissibility of *bean virus 2* through the seed is one of the chief points of difference between it and *bean virus 1*. The results of a test on transmission of *bean virus 2* through the seed of soybean is included in table 5. None of the seedlings was infected.

In one trial of 31 seedlings, grown from the seed obtained from Manchu soybean plants that had been artificially infected with *alfalfa virus 2*, none of the seedlings was infected. Gardner and Kendrick (15) described a mosaic of soybean, transmitted through the seed, but they were unable to infect varieties of *Phaseolus vulgaris* with their soybean-mosaic virus (23). It appears, therefore, that their soybean mosaic was not caused by *bean virus 2* nor by *alfalfa virus 2*.

The writer has made no tests on the seed transmission of tobacco ring-spot virus nor of *tobacco virus 1*. Transmission of a ring spot of tobacco through the seed of tobacco in a few instances was reported by Johnson (20). Valteau<sup>3</sup> reported that evidence had been obtained to show that tobacco ring spot was transmitted through tobacco seed. Henderson (16), although unable to confirm the report of Valteau, demonstrated that ring spot was transmitted through the seed of garden petunia to about 20 per cent of the seedlings. To the writer's knowledge no proof of transmission of *tobacco virus 1* through the seed of tobacco has been presented.

### Host Range

Each of the 5 viruses considered was tested on the following species of Leguminosae and Solanaceae: hyacinth bean, *Dolichos lablab* L.; lespedeza, *Lepedeza striata* Hook and Arn.; white lupine, *Lupinus albus* L.; common and Turkestan alfalfa, *Medicago sativa* L.; white sweet clover, *Melilotus alba* Desr.; black tepary bean, *Phaseolus acutifolius* Gray var. *latifolius* Freem.; adzuki bean, *Phaseolus angularis* Wight; mung bean, *P. aureus* Roxb.; rice bean, *P. calcaratus* Roxb.; scarlet runner bean, *P. coccineus* L.; Lima bean, Burpee's bush variety, *P. limensis* Macf.; Sieva Lima bean,

<sup>3</sup> Valteau, W. D. Tobacco seed beds in Kentucky. U. S. Dept. Agr. Pl. Dis. Rprtr. 14: 113. 1930. (Mimeogr.)

Henderson bush variety, *P. lunatus* L.; garden pea, Perfection variety, *Pisum sativum* L.; kudzu bean, *Pueraria hirsuta* Schneid.; soybean, Manchu and Mikado varieties, *Soja max* Piper; velvet bean, *Stizolobium* sp.; alsike clover, *Trifolium hybridum* L.; crimson clover, *T. incarnatum* L.; red clover, *T. pratense* L.; white clover, *T. repens* L.; broad bean, *Vicia faba* L. var. *minor*; asparagus bean, *Vigna sesquipedalis* Wight; cowpea, Groit variety, *V. sinensis* Endl.; tomato, Globe variety, *Lycopersicon esculentum* Mill.; tobacco, *Nicotiana tabacum* L. and *N. glutinosa* L. and garden petunia, Star of California variety, *Petunia hybrida* Vilm. The number of plants inoculated in each case varied from 5 to 25 or more. All inoculations were made artificially by the wiping method. Infection in hosts reported as susceptible was confirmed by inoculations to Refugee Green bean. By this procedure the presence or absence of any particular virus was ascertained, even though the inoculated plants did not show visible symptoms.

The reports of Reddick and Stewart (31) that *Phaseolus lunatus* var. *macrocarpus*, *P. acutifolius* var. *latifolius*, and *Vicia faba* and of Nelson (26), that *Phaseolus calcaratus* Roxb. and *P. lunatus* L. were susceptible to common bean mosaic were confirmed. Besides these hosts the mung bean, lespedeza, and spring vetch were found to be susceptible. Repeated inoculations with *bean virus 1* to Henderson Bush Lima bean gave only a small percentage of infection; it appears, therefore, that this variety is quite resistant to infection by this virus. The writer has been unable to confirm Nelson's (26) report that *Phaseolus angularis*, *P. coccineus*, and *Vigna sesquipedalis* are susceptible to common bean mosaic.

The host range of yellow bean mosaic (*bean virus 2*) parallels rather closely that of common bean mosaic. With the exception of Burpee's Bush Lima, *Phaseolus limensis*, Henderson Bush Lima, *P. lunatus*, and rice bean, *P. calcaratus*, all of the hosts of the latter were found to be susceptible to yellow bean mosaic. Hosts found to be susceptible to yellow bean mosaic and not to common bean mosaic were white sweet clover, soybean, white lupine, and crimson clover. Juice extractions of certain naturally infected sweet clover and soybean plants have been tested on bean and found to produce symptoms typical of yellow bean mosaic. In other transfers from mosaic sweet clover to bean, however, no infection of any kind was obtained, indicating that some virus not considered in this paper was concerned.

The host range of *alfalfa virus 2* is distinctly wider than that of the two bean viruses, as infection was obtained on certain solanaceous hosts as well as on a large number of leguminous hosts. In addition to certain varieties of common bean, the asparagus bean was found to develop local lesions after inoculation. Systemic infection was secured upon the follow-

ing: hyacinth bean, adzuki bean, mung bean, rice bean, common and Turkestan alfalfa, white sweet clover, crimson clover, red clover, white clover, Perfection pea, broad bean, spring vetch, Manchu and Mikado soybeans, garden petunia, and tobacco, *Nicotiana tabacum* and *N. glutinosa*.

It is beyond the scope of this paper to describe in detail the symptoms on all the hosts infected with different viruses. However, it should be mentioned that certain very definite differences were often apparent. For instance, soybeans infected with *alfalfa virus 2* exhibit certain characteristic symptoms differing in many respects from symptoms on soybeans infected with *bean virus 2*. Another example is that of broad bean, *Vicia faba*, on which infection with *alfalfa virus 2* results in a very pronounced vascular necrosis, finally killing the plants, while mottling of the leaves is the conspicuous symptom produced by *bean virus 2*.

Infection with *tobacco virus 1* was not secured on any of the legumes tested, except local infection upon certain varieties of *Phaseolus vulgaris*. The solanaceous hosts tested were all previously recognized hosts of this virus.

Tobacco ring-spot virus was found to infect a wide range of hosts. Priode (29) transferred ring spot to beet, *Beta vulgaris* L.; pokeweed, *Phytolacca decandra* L.; petunia, *Petunia hybrida*, and New Zealand spinach, *Tetragonia expansa* Murr. In a study of the host range of tobacco ring spot, Wingard (35) obtained successful transmission to 38 genera of plants, including transmission to the following Leguminosae: *Dolichos lablab*, *Melilotus officinalis* Lam., *Phaseolus lunatus*, *P. vulgaris* L., and *Vigna sinensis*. In the present study several other leguminous plants were found to be susceptible. The ring-spot virus produced only local necrotic lesions on the inoculated leaves of the following hosts: Henderson Bush Lima, *Phaseolus lunatus*, Burpee's Bush Lima, *P. limensis*, and for the most part of the varieties Corbett Refugee, Robust, and Mexican Tree of *Phaseolus vulgaris*. On many hosts local necrotic infection is followed by systemic infection, which in nearly all cases causes the death of the plants involved. Hosts thus affected were white lupine, Groit cowpeas, asparagus bean, soybean, broad bean, spring vetch, velvet bean, mung bean, rice bean, and many varieties of common bean.

The following hosts were found to develop systemic symptoms and did not develop visible local necrotic lesions at the points of inoculation: hyacinth bean, white sweet clover, crimson clover, and adzuki bean.

It is apparent that the host range of each of the five viruses here considered is different. A few of the more outstanding differences which may be useful in differentiating between them have been summarized in table 6.

## Separation of the Viruses Studied

The technique of separation of two or more viruses present in a single plant, on the basis of their host range and properties has been described by Johnson (21). The same principles may be employed in the separation of the 5 viruses considered in this paper. A summarized scheme for their differentiation and identification on the basis of hosts is presented in table 6.

The isolation of *bean virus 1* from mixtures with the other viruses is probably the most difficult to accomplish, since it is very sensitive to treatments of any kind. It may be separated from the others through the medium of the seed. By inoculating any virus mixture containing *bean virus 1* to the Mexican Tree variety, this virus would produce systemic infection, while *alfalfa virus 2*, *tobacco virus 1*, and tobacco ring-spot virus cause local infection. If *bean virus 2* were not present in the mixture *bean virus 1* could be obtained from the new growth showing mosaic symptoms. If *bean virus 2* were present it would be necessary to grow the plants to maturity and obtain *bean virus 1* from the seed.

*Bean virus 2* may be secured from a mixture with any of the other viruses by inoculation to Corbett Refugee, this variety being resistant to systemic infection by all the other viruses.

*Alfalfa virus 2* may be isolated from the other four viruses by inoculation to Perfection variety of pea, which is susceptible to the alfalfa virus but not to the other viruses. In case of a mixture with the 2 bean viruses, *alfalfa virus 2* may be separated from them by aging a mixed sample of the viruses for a period of 2 or 3 days. This length of time insures the inactivation of the 2 bean viruses but not that of the alfalfa virus.

The tobacco ring-spot virus may be secured free from any of the other viruses considered here by inoculation of cowpeas (Groit variety). Another method is to treat the virus mixture with 75 per cent alcohol for 30 minutes or with heat at 64° C. for 10 minutes. This inactivates all viruses except ring-spot and *tobacco virus 1*. The latter can be eliminated by inoculation of a variety of *Phascolus vulgaris* resistant to it, such as Red Valentine.

*Tobacco virus 1* in combination with any of the above viruses may be secured by inoculation of tomato, or by sufficient heating or aging to inactivate the other viruses in the mixture.

## DISCUSSION

The significance of the investigations presented in this paper is largely in the accumulation of evidence that bean and other legumes may be subject to a number of different viruses, and that these viruses may be readily differentiated or separated in various ways. It also is hoped that the description of certain new or previously undescribed viruses will be sufficiently

TABLE 6.—Summarized scheme for the differentiation and identification of bean virus 1, bean virus 2, alfalfa virus 2, tobacco virus 1, and tobacco ring-spot virus on basis of differential hosts<sup>a</sup>

Virus	Type of infection	HOSTS							
		<i>Phaseolus vulgaris</i>						<i>Pisum sativum</i>	<i>Vigna sinensis</i>
		Refugee Green	Corbett Refugee	Mexican Tree	Robust	Red Valentine	Perfection pea	Groit cowpeas	<i>Nicotiana glauca</i>
<i>Bean virus 1</i>	Local Systemic	— ++	— —	— ++	— —	— ++	— —	— —	<i>Nicotiana glauca</i>
<i>Bean virus 2</i>	Local Systemic	— ++	— +	— ++	— +	— ++	— —	— —	<i>Nicotiana glauca</i>
<i>Alfalfa virus 2</i>	Local Systemic	++ —	± —	± —	± —	++ —	— +	— —	<i>Nicotiana glauca</i>
<i>Tobacco virus 1</i>	Local Systemic	+ —	+ —	++ —	+ +	— —	— —	— +	<i>Nicotiana glauca</i>
<i>Ring-spot virus</i>	Local Systemic	± +	± —	+ ±	+ ±	++ ++	— —	+ +	<i>Nicotiana glauca</i>

<sup>a</sup> — indicates no infection; ± slight infection; + moderate infection; ++ severe infection.



complete to permit their definite recognition by future workers with legume viruses.

For purposes of preliminary comparison we have included in this study 3 viruses (*alfalfa virus 2*, *tobacco virus 1*, and the tobacco ring-spot virus), which are not known to affect the bean in nature. This fact should not be allowed to confuse the general understanding of the bean-virus problem itself. Besides the common bean-mosaic virus (*bean virus 1*) we have shown that another virosis of the bean occurs in nature, which has been named yellow bean mosaic (*bean virus 2*). That still other viruses may affect the bean is evident from Carsner's (6) transmission of the sugar beet curly-top virus to this host. Beyond this, however, the distinction between the above mentioned viruses and others reported to occur on bean and other Leguminosae becomes less apparent. Nelson's (26) rugose mosaic of the bean appears on the basis of transmission through the seed to be distinct from yellow bean mosaic, which it, symptomatically, most resembles.

The possible relationships and overlapping host ranges of bean viruses to those of other leguminous plants may be illustrated by the following cases. Transmission experiments with aphids led Merkel (24) to the conclusion that one and the same virus was responsible for the mosaic symptoms of all the Papilionaceae that he examined, which included *Phaseolus vulgaris*, *Pisum sativum*, *Lathyrus odoratus*, *Lupinus luteus*, *Melilotus altissima*, *Trifolium pratense*, *T. hybridum*, *T. repens*, *Anthyllis vulneraria*, and *Vicia faba*. Zaumeyer (36) transmitted to beans the viruses of mosaics of *Melilotus alba*, *M. officinalis*, *Trifolium repens*, *T. hybridum*, *Pisum sativum*, and *Lathyrus odoratus*. He stated that the virus of mosaic white sweet clover produced symptoms on beans slightly different from those of common bean mosaic. The virus concerned may have been identical with *bean virus 2* described in this paper.

There are, on the other hand, a number of reports of mosaics on leguminous plants that have not been connected with viruses affecting the common bean. Doolittle and Jones (8) described a garden-pea mosaic transmissible to sweet pea and red clover. They were, however, unsuccessful in producing the disease by inoculations from mosaic sweet clover, *Melilotus alba*, and mosaic bean, *Phaseolus vulgaris*, to garden peas and sweet peas. Böning (5) considered a virus disease of broad bean, which he found transmissible to red clover, crimson clover, and peas, as probably identical with the pea mosaic of Doolittle and Jones (8). The same virus may also have been concerned in the mosaic of *Trifolium pratense*, which Dickson (7) had previously shown to be transmissible to several species of *Trifolium* and to *Medicago lupulina* but not to *Melilotus alba* nor *M. officinalis*. Elliott (9), on the other hand, may have been concerned with another virus in his mosaic of sweet clover and red clover, since he found the virus to be transmis-

sible to the two latter hosts as well as to *Medicago arabica* and *Vicia faba*, but not to *Trifolium repens* nor *Medicago sativa*. As previously pointed out, the soybean mosaic of Kendrick and Gardner (23) was shown by them to be nontransmissible to bean; it would appear, therefore, that a specific virus, differing from any described in this paper, was responsible for their soybean mosaic.

It seems to be sufficiently evident from these investigations that further progress in and understanding of the viroses of the legumes as a whole or the virosis of any species in particular are dependent upon a more comprehensive study of the viruses themselves rather than of the diseases caused by them.

The existence in the bean of distinct immunity from and susceptibility to the common bean mosaic affords a promising field for the investigation of the inheritance of resistance to this disease, as well as for the development of new resistant commercial varieties. The fact that more than one mosaic disease exists necessarily complicates such a problem. Since no varieties of the common bean have been found that are entirely resistant to yellow bean mosaic, a continuation of the development of beans immune from common bean mosaic and moderately resistant to yellow bean mosaic would appear at present to offer the most promising means of eliminating losses from both diseases. It has been demonstrated that it is possible to secure families resistant to common bean mosaic from crosses of Corbett Refugee and Refugee Green. It is probable, therefore, that Corbett Refugee will be useful as a resistant parent variety for the improvement of many susceptible varieties of the canning type.

Since yellow bean mosaic (*bean virus 2*) is either not seed borne in the bean, or, if so, to only a slight extent, its chances of becoming of serious economic importance are greatly reduced. The fact that sweet clover has been found to be a host of *bean virus 2* suggests a means by which the virus probably overwinters. Isolation of bean plantings from infected sweet clover may prove effective in the control of yellow bean mosaic.

#### SUMMARY

The studies reported in this paper were concerned primarily with the resistance of varieties of *Phaseolus vulgaris* to the virus of common bean mosaic. In the course of the study other distinct viruses were found to affect the bean. To allow for the specific naming of such of them as had not been previously named an attempt has been made to differentiate the viruses on the basis of symptom expression, varietal susceptibility, properties *in vitro*, and transmissibility through the seed.

The susceptibility of 24 varieties of beans to the following viruses was determined by means of artificial inoculations in the greenhouse: common bean-mosaic virus (*bean virus 1*), yellow bean-mosaic virus (*bean virus 2*),

alfalfa-mosaic virus (*alfalfa virus 2*), tobacco-mosaic virus (*tobacco virus 1*), and tobacco ring-spot virus. *Bean virus 2* and *alfalfa virus 2* had not been previously described.

*Bean virus 1* and *bean virus 2* both produced systemic infection on beans, but the symptoms produced by *bean virus 2* were much more severe. *Alfalfa virus 2* and *tobacco virus 1* caused only local necrotic infection; and the tobacco ring-spot virus caused both local and systemic infection on beans. Symptoms on differential varieties were described.

The bean varieties were grouped according to symptom expression and susceptibility to *bean virus 1* as either: I, susceptible; II, tolerant; or III, immune. In general the same grouping held for reaction to *bean virus 2*, although none of the varieties were found to be immune from this virus. The susceptibility to the 2 bean viruses was not correlated with susceptibility to the other viruses studied.

The thermal inactivation point, resistance to aging *in vitro*, tolerance to dilution, and resistance to certain chemicals were determined for all the viruses except *tobacco virus 1*; the properties of the latter have been previously determined by Allard and others. The thermal death point of *bean virus 1* and *bean virus 2* was found to lie between 56° and 58° C. for 10 minutes; of *alfalfa virus 2* between 62° and 64° C.; and of tobacco ring-spot virus at 66° C. In aging-*in-vitro* experiments, *bean virus 1* and *bean virus 2* lost their infectivity after 24 to 32 hours; *alfalfa virus 2* and tobacco ring-spot virus in from 7 to 9 days. The infectivity of all the viruses was either lost or greatly reduced at dilutions greater than 1-1000. Treatments with alcohol, nitric acid, and formaldehyde showed minor differences in the resistances of the viruses to these treatments.

Previous reports of transmission of common bean-mosaic virus by the potato aphid, *Macrosiphum solanifolii*, and by the pea aphid, *Illinoia pisi*, were confirmed. *Bean virus 2* was also shown to be transmitted by these aphids. *Alfalfa virus 2* was readily transmitted by the pea aphid.

In tests involving 6532 seedlings no evidence of transmission of *bean virus 2* through the seed was obtained. *Bean virus 1* was transmitted through the seed, as previously reported.

The host-range studies showed important differences between the viruses studied. Sweet clover was found to be susceptible to *bean virus 2*, and it was suggested that this host may serve to overwinter this virus.

While specific viruses other than those described in this paper play a part in the virus infections occurring on leguminous plants, it is hoped that the data presented in this paper will be of value in the ultimate differentiation of all the viruses affecting plants of the Leguminosae.

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# RESISTANCE OF POTATO TO MOSAIC AND OTHER VIRUS DISEASES<sup>1</sup>

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## INTRODUCTION

The use of resistant varieties of plants is the most effective method of disease control. Certain potato diseases, such as late blight, can be controlled to some extent by spraying but in years of severe epidemics they cause heavy losses in spite of control measures. Other potato diseases can be partially controlled by seed treatment and by soil disinfection. Potato viroses, however, cannot be controlled by these methods.

At present the most effective methods for the control of a potato virosis are tuber indexing, early and thorough roguing, and early harvesting of isolated tuber-unit seed plots, together with the importation of seed from a region in which spread from plant to plant is generally infrequent, or the use of an immune, resistant, or tolerant variety. The last method of control has been applied to mild mosaic in commercial practice to the extent that the resistant Irish Cobbler and Spaulding No. 4 (Syn. Rose No. 4) varieties are grown. Proof of this resistance and the development of other resistant varieties are described in this paper, together with experiments with other potato viroses.

## DESCRIPTION OF DISEASES

Mild mosaic is manifested on the variety Green Mountain by slight wrinkling, ruffling, and dwarfing and by distinctly light green spots more or less indistinctly delimited by irregular margins from the dark green leaf areas. Next to latent mosaic, or seedling streak, mild mosaic is found most commonly in Green Mountain in the important seed-potato localities.

Leaf roll is manifested by rolling and rigidity of the leaves and by distinct dwarfing and chlorosis. Tubers of leaf-roll plants usually are sessile and reduced in size and number.

Spindle tuber is manifested by more or less spindling, erect, slightly rugose plants, which are dwarfed and somewhat darker green than healthy tops. Spindle-shape tubers also distinguish this malady from other viroses.

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Although our observations were concerned primarily with resistance of potato varieties and seedlings to mild mosaic, leaf roll, and spindle tuber, less extensive studies also were made on resistance of seedlings to streak, which also has been called stipple streak, or acropetal necrosis, and to seedling streak, or top-necrosis.

Streak is characterized on Green Mountain in current season infections by necrotic streaking and spotting on leaves, petioles, and stems and by brittleness, leaf fall, and premature death. Second-season symptoms are manifested by severe dwarfing, necrotic spotting and streaking, and premature death, which usually precedes tuber formation.

Top-necrosis, or acronecrosis (Quanjer 10), frequently has been designated as seedling streak in our work, since it was encountered in connection with studies on the reaction of different potato seedlings to mosaic and other viroses. Inasmuch as Green Mountain was found to be a latent carrier of top-necrosis, this disease also has been called latent mosaic. As the name of this disease indicates, it involves at first the apical part of the plant where it causes severe necrosis on leaf, petiole, and stem. Necrosis appears in cortex and pith of stems, as well as in tubers. Eyes of tubers may be killed or partly infected so that necrotic as well as apparently healthy shoots may develop from such tubers during the season that follows the one in which infection occurred. Acronecrosis is not generally so superficial as streak, or acropetal necrosis.

#### METHODS OF INOCULATION USED IN TESTING PLANTS FOR RESISTANCE

Plants were exposed to infection by two methods: (1) Healthy plants were planted either in hills alternating with hills of diseased plants or in rows alternating with rows of diseased plants. (2) Healthy tubers were grafted with diseased tubers by means of splice or plug grafts. The Green Mountain variety served as the source for diseased plants and as a healthy control. Since symptoms of infection are not always discernible in the season in which the infection takes place, the tubers from the exposed plants were grown the next season and disease data secured.

In the field tests during 1924 to 1930 25 hills of each variety or seedling to be tested were planted alternately with hills of diseased material. Three series were grown, 1 for each of the 3 diseases, mild mosaic, leaf roll, and spindle tuber.

In 1931, the seedlings were planted in 10-hill lots, except in a few lots of the 2 naturally fertilized progenies where sufficient seed stock was not available for 10 hills. These lots were planted consecutively in rows alternating with Green Mountain potatoes affected with mild mosaic. Ten-hill lots of apparently healthy Green Mountain seed pieces were planted as controls in representative parts of each seedling row.

Most of these experiments were conducted at Presque Isle, Maine, where conditions usually favor the expression of mosaic symptoms. Some of the tuber grafts were made and planted in the greenhouse at Arlington Farm, Virginia.

*Field Exposure of Potatoes to Mild Mosaic, Leaf Roll, and Spindle Tuber, 1924-1930.* Thirty-three potato seedlings representing 18 crosses were exposed to Green Mountain potatoes affected with mild mosaic under field conditions in northern Maine in 1924-1930. During the first 4 years of these tests, 1924-1927, 2 tubers per hill were saved for the following season's planting, but from 1928 to 1930, inclusive, 3 tubers were harvested for this purpose.

*Tuber-graft Inoculations.* In the tuber-graft inoculations usually 8 to 10 tubers of a variety or seedling were plug- or splice-grafted with diseased tubers. A seed piece of each of the healthy and the diseased tubers was planted as a control. In most of these inoculations the Green Mountain variety represented the source of the diseased material.

Johnson (4) and Schultz (12) reported that certain potato varieties, like the Green Mountain, were susceptible to mild mosaic and at the same time were masked carriers of latent mosaic, resulting in mixed or composite infection. In the early years of the experiments here reported seedlings were produced by hybridization and selection, some of which were susceptible to both mild and latent mosaic, giving the same complex as that found in Green Mountain, others that were somewhat susceptible to the mild but resistant to the latent form, and still others that were resistant to the mild mosaic but susceptible to the latent. In this last group the latent form often expresses itself as severe streak or necrosis. The seedlings that are resistant to latent mosaic simplify the work since they make it possible to separate the mild form from the latent type. Accordingly, in 1931 and 1932, a seedling that becomes infected with mild mosaic but is resistant to latent mosaic was used instead of Green Mountain as the source of mild mosaic in the tuber-graft tests.

#### REACTION OF POTATO TO LEAF ROLL AND SPINDLE TUBER

From 1924 to 1930, 33 potato seedlings and Green Mountain, Irish Cobbler, and Spaulding No. 4 were exposed to spindle tuber. In 1924, 1925, 1928, 1929, and 1930, 18 potato seedlings also were exposed to leaf roll, under the same method used for exposing these potatoes to mild mosaic. The results recorded in table 1 disclose that nearly every seedling and variety became infected with these diseases. No apparent differences in severity of symptoms of leaf roll and spindle tuber were observed between these selections. However, some of the seedlings manifested spindle tuber on the tubers in the form of pear-shape tubers instead of spindle-shape



tubers, as commonly found in Green Mountain. A few seedlings apparently reacted to spindle tuber by manifesting slightly rolled leaves in addition to the erect and more or less spindling tops, which are characteristic symptoms of spindle tuber.

Leaf roll and spindle tuber spread much more generally in 1929 than during the other seasons. Inasmuch as most of the seedling lots were infected with spindle tuber when they were introduced to the trial plots, the percentage of spindle tuber recorded in table 1 represents only a part of the infection contracted in the exposure tests. Since spindle tuber is spread by means of the cutting knife, it is possible that this method of infection also contributed to the results.

Experience with leaf roll and spindle tuber has shown that these maladies usually are spread less widely in northern Maine than mild mosaic. Consequently, field exposure tests with mild mosaic in this locality have been more reliable than similar trials with leaf roll and spindle tuber.

#### REACTION OF POTATO TO STREAK

Observations on the reaction of Spaulding No. 4 and Green Mountain to streak have disclosed that Spaulding No. 4 manifests the symptoms of this disease in milder form than Green Mountain. Although Spaulding No. 4 develops necrotic spots and streaks on leaves, stems, and petioles, these symptoms are not so conspicuous as those on Green Mountain. Spaulding No. 4, affected with streak, produces a fair crop of tubers, while streak-affected Green Mountain tops from streak-infected tubers are so severely dwarfed, streaked, and curled that tuber development usually is inhibited.

In 1931, tubers from several apparently healthy potato varieties were tuber-grafted on apparently healthy Green Mountain tubers. The Green Mountains tuber-grafted to one of these varieties developed typical symptoms of streak, which indicates that this variety is a latent or masked carrier of this disease. Atanasoff (1) pointed out that he found certain varieties that were masked carriers of streak.

#### REACTION OF POTATO TO LATENT MOSAIC

The term latent mosaic is not used here to designate a single virus but it may include several viruses the symptoms of which are not manifested in some varieties. It is harbored in masked or latent form by Green Mountain, Irish Cobbler, Bliss Triumph, and other commercial varieties. Some of these varieties or strains of them have very few plants that are free from latent mosaic.

Among the commercial varieties tested none has been found that does not carry latent mosaic in one form or another in a high percentage of plants. A seedling, U. S. D. A. number 41956, has been produced, however,

that has shown resistance to latent mosaic. Leaf rubbing and tuber-graft inoculations with this virus on this seedling, which has been tested for several years, have so far failed to induce infection. Comparable inoculations on Green Mountain seedlings free from latent mosaic resulted in every plant becoming infected with this disease.

In field tests seedling 41956 has been grown for several seasons in contact with latent-mosaic Green Mountain but has not contracted the disease in a single case. On the other hand, latent-mosaic-free Green Mountain seedlings, similarly propagated, contracted latent mosaic in 15 per cent of the plants in a single season. Since leaf-rubbing, tuber-grafting, and field-contact tests have failed to produce infection of latent mosaic in seedling 41956, it is highly resistant to this virus under all conditions involved in the tests. Schultz and Raleigh (14).

Graft and juice inoculations from masked carriers of latent mosaic to certain other varieties or seedlings result in severe necrosis on tops and tubers. This necrosis has been designated top-necrosis or acronecrosis. (Quanger 10). Seedlings showing this reaction have been found that seldom contract infection when exposed to latent-mosaic Green Mountain in the field. If these are compared with the Green Mountain seedlings noted above, which contracted latent mosaic in 15 per cent of the plants in a single season, they can be considered resistant to latent mosaic in field tests. Under the more severe tests of tuber grafting and juice inoculation, top necrosis developed. While it is highly desirable to produce varieties resistant to this or any disease, under all conditions, it is, nevertheless, important from the standpoint of breeding commercial varieties to produce seedlings resistant to a disease when grown under the usual field conditions. As the vector for latent mosaic has not been found, it is impossible to specify the factors that may be responsible for the differences in the response of these seedlings to latent mosaic under these conditions. However, since latent mosaic can easily be transmitted by leaf rubbing, it appears very possible that contact of diseased with healthy plants in the field may transmit latent mosaic and that such transmission may occur more readily in some varieties than in others. In connection with their investigations on necrotic viroses of potato, Salaman and Bawden (11) point out that the evidence is suggestive of the existence of a definite resistance to certain viruses in the variety Abundance. Among these viruses was included a virus or virus complex that apparently is identical with the one involved in latent mosaic.

#### REACTION OF POTATO TO MILD MOSAIC

Experience with Irish Cobbler has shown that this variety apparently fails to react to mild mosaic. Leaf-rubbing and tuber-graft inoculations from mild-mosaic Green Mountain to Irish Cobbler have apparently failed

to infect this variety. Likewise, return or back grafting of tubers from Irish Cobblers inoculated with mild mosaic to apparently healthy Green Mountains has failed to produce infection. Inasmuch as more than a hundred such tuber grafts have been made at various times and planted in field and under greenhouse conditions without producing mild mosaic on Irish Cobbler, and since this variety has failed to manifest symptoms of mild mosaic after having been grown for several seasons next to mild-mosaic Green Mountain, it is without doubt resistant to mild mosaic. However, Irish Cobbler, like Green Mountain, is a symptomless carrier of latent mosaic and clearly manifests the same reactions to rugose mosaic, acropetal necrosis, and other potato viroses, as does Green Mountain.

Our experience indicating that the Irish Cobbler variety manifests resistance to mild mosaic has been confirmed by seed-potato growers who find that leaf roll, spindle tuber, and rugose mosaic are the diseases they must eliminate from this variety in order to maintain it free from viroses. Growers frequently have propagated Irish Cobbler adjoining mild-mosaic Green Mountain, with the result that the Irish Cobbler failed to contract mild mosaic, while healthy Green Mountain similarly propagated developed more than 50 per cent of this disease during a single season.

The differences observed between varieties and seedlings with respect to their reaction to mild mosaic were the basis of a systematic breeding program, which was started for the purpose of producing new seedling varieties in which it was sought to combine resistance to this disease with other desirable commercial qualities. Careful observations were made of the most fertile seedlings and data secured on the amount of mosaic found when grown under the conditions prevalent in the plant-breeding plots for a series of years. Two of these, S. 24642 and S. 40568, were comparatively free from mild mosaic for a number of years. For the 3 years 1918, 1920, and 1921, S. 24642 was free from mild mosaic, and in 1919 it showed approximately 2 per cent. For the same period of time, 1918-1921, S. 40568 produced one mild-mosaic plant out of approximately 500 plants grown. In comparison with this, S. 39477, one of the susceptible seedlings, produced no mild mosaic in 1919, 2.7 per cent in 1920, and 49.7 per cent in 1921. From the data available in 1921, then, S. 24642 and S. 40568 were apparently resistant to mild mosaic. Consequently a large number of crosses were made in which these seedlings were used as parents. These 2 seedlings were crossed with each other and with other seedlings and varieties.

As the main object of the early breeding work was to obtain varieties superior in many respects to the commercial varieties only those seedlings were selected from the progenies of these crosses that were desirable from the standpoint of shape, shallowness of eye, yield, and other characters of economic importance. In the cross between the 2 seedlings, S. 24642 and

S. 40568, 40 selections were made in 1923. A number of these were found to be more susceptible than either of the parents under field conditions, since 6 of them developed 100 per cent mild mosaic in one year, 1924. On the other hand, 2 selections of this progeny, S. 42667 and S. 42672, grown under the same conditions, have shown a higher degree of resistance than either of their parents. These 2 seedlings and 31 others, representing in

TABLE 1.—*Reaction of certain potato varieties to viroses. Potatoes exposed to infection in the field, Aroostook Farm, Presque Isle, Maine—1924–1930*

Variety	Year	Total hills	Symptoms and hills infected					
			Mild mosaic		Leaf roll		Spindle tuber	
			No.	Per cent	No.	Per cent	No.	Per cent
Green Mountain	1924	50	20	40			5	10
	1925	50	22	44	5	10	3	6
	1926	50	42	84			2	4
	1927	50	23	46			3	6
	1928	75	68	91	4	5	1	1
	1929	75	72	96	46	61	46	61
	1930	75	29	39	2	2	10	14
Irish Cobbler	1924	50					22	44
	1925	50			3	6	2	4
	1926	50					5	10
	1927	50					3	6
	1928	75					6	8
	1929	75			50	66	48	64
	1930	72					15	20
Spaulding No. 4	1924	50					14	28
	1925	50			10	20	8	16
	1926	50					2	4
	1927	50					2	4
	1928	75			5	7		
	1929	75			38	51	47	63
	1930	75					48	66
Jersey Red Skin	1926	50	23	46			11	22
Early Ohio	1925	50	8	16	7	14	23	46
24642	1924	50	13	26	2	4	8	16
	1925	50	5	10			5	10
41420	1925	50	4	8	14	28	12	24
41499	1926	50	4	8			20	40
41558	1925	50	2	4			3	6
41803	1925	50	8	16	4	8	40	80

TABLE 1.—(Continued)

Variety	Year	Total hills	Symptoms and hills infected					
			Mild mosaic		Leaf roll		Spindle tuber	
			No.	Per cent	No.	Per cent	No.	Per cent
41865	1925	50	7	14			21	42
41881	1926	50	4	8			11	22
41914	1924	50	10	20			12	24
41914	1930	75					14	18
41916	1924	50	8	16			13	26
41917	1924	50	2(†)	4	11	22	6	12
41918	1926	50	7	14			4	8
41956	1925	50	3	6	4	8	25	50
41956	1927	50	7	14				
41956	1930	75	1	1	4	5	13	17
41977	1925	50	12	22			10	20
41988	1926	50	13	26			11	22
42016	1926	50	12	22			3	6
42667	1929	75			22	29	30	40
42667	1930	75					18	24
42672	1929	75			67	89	66	88
42672	1930	75			6	8	27	36
42857	1927	50	3	6				
42857	1928	75					9	12
42858	1928	75	4	5	6	8		
42898	1927	50	4	8			10	20
42901	1928	75	1	1	1	1		
42903	1928	75	12	16	9	12	3	4
42906	1927	50	10	20			2	4
42949	1927	50	5	10			8	16
42953	1927	50	7	14			3	6
42963	1928	75	1	1	10	13	13	17
42966	1928	75	4	5	4	5	9	12
42967	1928	75	1	1	5	7		
43011	1927	50	9	18			2	4
43106	1927	50	3	6			4	8
43313	1928	75	1	1	12	16	9	12
43325	1928	75	20	27	10	13		
43327	1928	75	12	16	25	33		

all 18 different crosses, were subjected to more severe tests than those that prevailed in the plant-breeding plots. These 33 seedlings were grown in proximity to Green Mountain plants infected with mild mosaic. These tests extended over the period of years from 1924 to 1930, inclusive. Six of these were exposed during 2 or more seasons, but the remaining 27 were exposed during one season only. Table 1 gives the data for these tests.

The two resistant seedlings, S. 42667 and S. 42672, failed to develop mild mosaic in this test, although their parent, S. 24642,<sup>7</sup> gave an average of 18 per cent for the two years 1924 and 1925. The percentage of mosaic in the remaining 30 seedlings varied from 1 to 27. Most of these contracted less than 10 per cent mosaic, while the Green Mountain checks developed from 39 to 96 per cent of mild mosaic. A few of the seedlings manifested symptoms of mild mosaic like those in Green Mountain, but in most of them the presence of this disease was indicated by faint mottling and slight wrinkling or rugosity or by symptoms that are less pronounced than those in mild-mosaic Green Mountain. It is possible that these faint mosaic symptoms may be due partly to the absence of latent mosaic. On the other hand, these faint mosaic symptoms may be the expression of mild mosaic in a seedling that is partly resistant to the virus. None of the potato seedlings in the test reported here developed mosaic in as many plants as the Green Mountain controls, and only a few of them manifested symptoms of mild mosaic like those in Green Mountain. It should be pointed out here that the seedlings reported in table 1 were the apparently resistant selections made in the plant-breeding plots and that many of the sibs of these seedlings were extremely susceptible, some of them producing 100 per cent mosaic plants in the plant-breeding plots in the second year after being grown from true seed. Seedling 42667, a selection from the progeny of the cross between the two resistant seedlings 24642 and 40568, is very highly resistant to mild mosaic. It has been grown in the plant-breeding plots for 10 years entirely free from this disease. During the same time many other seedlings, grown under the same conditions, produced 100 per cent mild mosaic. It remained free from mild mosaic after it had been grown adjoining mild-mosaic Green Mountain in the disease test plots for 3 seasons. During this period Green Mountain controls carrying latent mosaic developed mild mosaic in 80 per cent of the plants. Seedling 42667 has been subjected to the more severe tests of tuber grafting, with results that again show a high degree of resistance to mild mosaic.

In 1930 this seedling was tuber-grafted on mild-mosaic Green Mountain. Splice and plug grafts were made. In the plug grafts 2 plugs of a diseased tuber were inserted into the healthy tuber, except in series 6, table 2, in which healthy plugs were inserted into the diseased tuber from which the eyes had been removed. The healthy as well as the mild-mosaic Green Mountain carried latent mosaic. Since S. 42667, grafted on latent-mosaic Green Mountain, developed top necrosis, as shown in series 1, later grafts were made on mild-mosaic seedling 41956, which did not carry latent mosaic. The results of these grafts are recorded in table 2.

<sup>7</sup> In the breeding plots that represent ordinary field conditions the average per cent of mild mosaic found in this seedling for a period of 12 years was 1.4.

TABLE 2.—*Reaction of seedling 42667 to mild mosaic. Tuber-graft inoculations, Presque Isle, Maine, 1930-1932*

Series	Graft	Number plants infected	Symptoms
		Number tubers grafted <sup>a</sup>	
1	S. 42667 on mild-mosaic G. M. <sup>b</sup>	3/8	Tip necrosis on S. 42667 Mosaic type? G. M. Mottled (mosaic type?) <sup>c</sup> non-graft control S. 42667
2	Healthy G. M. on S. 42667 showing top necrosis in Series 1	1/9	
3	Healthy G. M. on S. 42667 showing few mottled areas	0/5	
4	S. 42667 on mild-mosaic 41956	0/13	Light-green patches on S. 42667 (mosaic type?) Mild-mosaic G. M.
5	Do	0/20	
6	S. 42667 plugs inserted into mild-mosaic 41956 tubers	2/10	
7	Healthy G. M. on mild-mosaic 41956	9/20	
8	Healthy G. M. on S. 42667 exposed to mild-mosaic G. M. in field during 2 seasons	0/100	

<sup>a</sup> The numerator indicates the number of plants infected, and the denominator the number of tubers grafted.

<sup>b</sup> G. M.—Green Mountain.

<sup>c</sup> Inoculations with this type of mosaic made by the writers after the manuscript of this paper was submitted indicate that this mosaic type may be the same as latent mosaic.

Since top necrosis was the only reaction manifested by S. 42667 in series 1, the tuber progeny of the 3 hills showing top necrosis were reserved for back or return grafts on healthy Green Mountain. The results of these back grafts are recorded in series 2, which shows that one of the 9 grafted tubers developed mosaic. The S. 42667 plants from the nongrafted control tubers in series 2 manifested slightly rugose leaves with distinctly mottled spots. Since additional tuber grafts with these mottled S. 42667 on healthy Green Mountains, as indicated in series 3, yielded no apparent infection, it is not possible to say what type of mosaic the mottling on S. 42667 here represents. Additional inoculations with this mosaic type are in progress to determine its identity.<sup>8</sup>

As disclosed in series 4 and 5, which represent 32 tuber grafts of S. 42667 on mild-mosaic 41956, no evidence of infection resulted. Likewise, in

<sup>8</sup> Inoculations with this type of mosaic made by the writers after the manuscript of this paper was submitted indicate that the mosaic type may be the same as latent mosaic.

series 8, involving tuber grafts of healthy Green Mountains on S. 42667, including a random sample of 100 tubers from tops grown adjoining mild-mosaic Green Mountains, no mild mosaic resulted, while in series 7 the control graft of healthy Green Mountain on mild-mosaic 41956, 9 in 20 tubers, or 45 per cent of the grafted tubers, developed mild mosaic. Although S. 42667 developed mosaic symptoms (type not determined) besides top necrosis in a few of the tuber grafts, these results indicate that this seedling manifests resistance to mild mosaic in the tuber grafts also.

In 1931, a naturally fertilized progeny of S. 42667 was grown in the disease test plots between rows of mild-mosaic Green Mountain plants. Although this progeny was produced from seed that resulted from natural fertilization, it is safe to assume that a very high percentage of the seed was the result of self fertilization or its equivalent, since the fruits were obtained in a comparatively large plot of this seedling with no other good pollen-producing varieties in close proximity. Four hundred and sixty-four selections of this progeny were grown in this test. At harvest 3 tubers from each hill were reserved for planting and observation the following season. Table 3 gives the data obtained.

It will be seen that only 7 per cent of these seedlings showed mild mosaic. It will be remembered that S. 42667 resulted from a cross between 2 seedlings, S. 24642 and S. 40568, both of which were apparently resistant to mild mosaic under ordinary field conditions. In the same field-contact test of 1931, a naturally fertilized progeny of seedling 43752, which had only

TABLE 3.—*Potato seedlings and Green Mountain, exposed to mild-mosaic Green Mountain in field test plots. Presque Isle, Maine, 1932*

Parentage of seedlings	Variety	Lots			Hills in mosaic lots		
		Total	Mosaic		Total	Mosaic	
			<i>No. Percent</i>			<i>No. Percent</i>	
S. 42667 naturally fertilized	464 seedlings	464	33	7	842	138	16
S. 43752 naturally fertilized	75 seedlings	75	21	28	550	101	18
S. 42672 × S. 42667	101 seedlings	101	27	27	754	119	16
S. 43322 × S. 42667	77 seedlings	77	34	44	971	192	20
	Green Mountain controls	54	54	100	1584	1061	67



one resistant parent, *viz.*, 40568, was grown. Twenty-eight per cent of the selections from this progeny showed mild mosaic. A small progeny of a cross between 2 resistant sibs, S. 42672 and S. 42667, was also included in these tests. Twenty-seven per cent of the seedlings in this progeny showed varying degrees of infection with mild mosaic in 1932. (Table 3). Another progeny in these tests was that of a cross between a parent that developed a few mild-mosaic plants in the plant-breeding nursery, S. 43322, and the most resistant parent yet available, S. 42667. It will be seen from the data in table 3 that this progeny does not show the same degree of resistance as the other progenies. In this case 44 per cent of the seedlings were infected with mild mosaic. These progenies will be given another test before final conclusions are drawn. The results, however, are quite significant when a comparison is made between the percentage of seedlings of each of the progenies that contracted mild mosaic and the control plots of Green Mountain. The data in table 3 show that every plot of these Green Mountain controls developed mild mosaic. It is evident, then, that a high percentage of the seedlings in each progeny tested is more resistant than Green Mountain in field tests.

#### DISCUSSION

The data for the tests of leaf roll and spindle tuber are not conclusive, as seasonal variation in the spread of these 2 diseases in the Green Mountain checks grown under field conditions is so variable. The results show, however, that most of the seedlings are susceptible to both of these diseases. A few of them show a low percentage of infection for the years in which they were grown, but it will be seen that the Green Mountain checks for the corresponding years also showed a similar condition, so that the low degree of infection can not be considered an indication of resistance.

Varieties differ in their reaction to streak. Spaulding No. 4 manifests the symptoms of this disease in a milder form than Green Mountain. According to Atanasoff (1), some varieties are masked carriers of streak. Since differences in reaction such as those noted exist, it ought to be possible to produce varieties that will be very resistant to this disease.

The reaction of different potato varieties to streak may be grouped as follows: (a) Latent or masked carriers. (b) Varieties manifesting mild necrosis on the foliage. (c) Varieties manifesting severe dwarfing, curling, and necrosis on tops that usually die before tubers are developed.

Field tests with 2 other viroses, *i.e.*, latent mosaic and mild mosaic, have shown that wide differences exist between varieties in their reaction to each of these diseases. The Green Mountain variety is quite susceptible to both of these diseases under conditions prevalent in northern Maine. One seedling, S. 41956, has been produced that is quite resistant to latent mosaic. Field-contact tests, leaf rubbing, and tuber grafting failed to produce a plant carrying this virus. It is, however, susceptible to mild mosaic but

fewer of the plants contract the disease than is true of the variety Green Mountain. Another seedling, S. 42667, is extremely resistant to mild mosaic and rarely contracts latent mosaic in the field tests, but it becomes infected with latent mosaic in the more severe tests when the disease is expressed in the plant as top-necrosis. Others are found that are as easily infected as the Green Mountain.

The reaction of the different potato varieties and seedlings to latent mosaic may, therefore, be grouped under three heads, (a) varieties highly resistant to latent mosaic as S. 41956, (b) varieties resistant in field tests but contracting latent mosaic in tuber and shoot grafts and manifesting the disease as top necrosis as S. 42667, (c) symptomless or masked carriers. Although varieties like S. 42667 readily develop top necrosis in tuber- or shoot-graft inoculations, they rarely contract this disease in contact with symptomless carriers under field conditions. It has been found that latent-mosaic carriers like Green Mountain seedlings contract latent mosaic more easily, apparently through contact with diseased foliage, than do seedlings manifesting this disease as top necrosis. It is probable that in the presence of the insect vector, yet to be found, latent mosaic will be a serious factor in varieties manifesting it as top necrosis. Otherwise, such seedlings may be propagated with the same degree of success as the highly resistant group and remain essentially free from latent mosaic, even if grown in contact with masked carriers of the disease. This statement is supported by the fact that the seedling cited as an example of the group that develops latent mosaic as top necrosis has been grown for 10 years in the plant-breeding plots. During this time not a single case of top necrosis has developed in spite of the fact that in one year, at least, it was grown in a row adjoining a Green Mountain row.

The reaction of different potato varieties and seedlings to mild mosaic may be grouped under the following heads: (1) Varieties or seedlings highly resistant to mild mosaic in all tests. (2) Seedlings that seldom contract the disease in the field but become infected as the result of tuber grafts. (3) Seedlings that become infected but manifest milder symptoms than those found on Green Mountain. (4) Seedlings that contract the disease as readily as Green Mountain and express symptoms similar to those found in mild-mosaic Green Mountains. These differences between seedlings are plainly evident but the number of factors responsible for them is not so clearly defined. Green Mountain has been used as the source of mild-mosaic inoculum, and, since most Green Mountain plants harbor a complex of 2 or more viruses, the results are somewhat complicated. Some of the seedlings tested for mild mosaic are possibly resistant to latent mosaic carried by the Green Mountain and, therefore, show milder symptoms. In field tests many seedlings may escape infection, and, yet, mild mosaic may be transmitted to them by means of tuber grafts. It is probable that such

seedlings harbor morphological or physiological characters that in some manner interfere with infection or inoculation by insect vectors. Whatever the factors involved may be, it is apparent that such seedlings can be maintained free from mild mosaic with considerably less effort than Green Mountain in northern Maine.

It is quite evident that some of the factors involved are genetic in nature and that resistance and susceptibility to mild mosaic are heritable. This conclusion is substantiated by the results obtained from a cross between 2 seedlings resistant to mild mosaic in the field. In the  $F_1$  progeny seedlings were obtained that were more resistant to this disease than either parent, and others that were more susceptible. One of the highly resistant seedlings of this progeny, No. 42667, was inbred and an  $F_2$  progeny was obtained. Four hundred sixty-four of these seedlings were tested for their reaction to mild mosaic in 1931 and 1932. The 1932 data show that about 7 per cent of these seedlings became diseased, while, under the same conditions, healthy Green Mountains planted as checks contracted the disease in 100 per cent of the plots. Two or more genetic factors, cumulative in effect, are necessary to interpret these results. It should be pointed out here, however, that the number of plants of some of these seedlings was less than those grown in the Green Mountain control plots in 1930. For this reason a number of the seedlings may have escaped infection. Further tests will be made to determine this point.

In the early years of breeding for resistance to mild mosaic the main object was to produce varieties of economic importance that possessed resistance to this disease; therefore, only the selections that were apparently resistant to the virus in the plant-breeding plots were given further tests in the disease test plots. Here they were grown in contact with mild-mosaic Green Mountains or given the more severe tests, such as tuber grafting, etc. As a result, the data from 1924 to 1930 are rather fragmentary. Beginning with 1931, however, fewer progenies have been tested in the plant-disease plots but larger numbers of each progeny have been grown. In these cases the seedlings were not previously selected for resistance or susceptibility to the virus disease.

It might be mentioned here that the first objective of this program, *viz.*, the production of varieties of potatoes of economic value resistant to mild mosaic, has been accomplished. One of these, S. 42667, which has been named Katahdin (3) has been given extensive tests in a number of States and to a considerable extent it has been distributed to commercial growers through the cooperating experiment stations.

Our experience has shown that, although certain potato seedlings may readily be infected with mild or latent mosaic by means of tuber grafts, nevertheless, these same seedlings apparently are resistant to infection under field conditions. In connection with this experience it may not be

amiss to recall the common experience with leaf roll of potato. It is well known that attempts to transmit leaf roll to healthy potatoes by the leaf-rubbing method of inoculation have failed. Certain aphids, however, readily transmit this disease. It then would seem reasonable to suppose that certain varieties could easily be infected by artificial methods, but would resist infection from insect vectors.

Artificial methods of infection give, among other things, valuable information regarding immunity, symptoms, conditions favoring infection, etc., but such methods are not necessarily infallible criteria regarding the reaction of plants to disease under natural field conditions.

It also has been indicated that potatoes may be resistant to infection from insect vectors but not necessarily to artificial methods of infection, such as tuber or shoot grafting.

It is realized that these investigations on resistance of potato to mosaic were not conducted with pure cultures of viruses. The Green Mountain variety, the source of infection for the field-exposure tests and for many of the tuber grafts, harbored latent mosaic in combination with mild mosaic. The "healthy" Green Mountain controls carried latent mosaic in masked form. However, in some of the inoculations seedlings carrying only mild mosaic, which, in combination with latent mosaic, produced the typical mild-mosaic symptoms on Green Mountain were used. Preliminary observations indicate that mild mosaic and latent mosaic may be caused by more than two viruses.

The recent work of Burnett and Jones (2), Johnson (5), Koch (6), Murphy and McKay (8), Quanjer (10), Smith (15), Salaman and Bawden (11), and Valleau and Johnson (16) has confirmed the former contention and findings by Murphy (7), Quanjer (9), and Schultz and Folsom (13) that there are several types of mosaic of potato and has extended these findings, showing by analysis and synthesis that more than one virus is involved in potato mosaic. Although it may be difficult to develop a potato that is resistant to all of the reported viruses, our results show genetic segregation for resistance to certain of the mosaics and we may conclude that it is possible to produce varieties resistant to many if not all of these diseases.

From the evidence of several viruses and possible virus complexes that may infect the potato and from the possible menace of masked carriers of viruses to nonresistant varieties, the potato grower might have adequate grounds for concern about the fate of this important crop. Fortunately, however, potato localities vary regarding the presence and spread of virus diseases; it is uncommon to find all the reported viruses in a single potato section, usually only a few of them requiring serious attention. In northern Maine, mild mosaic in Green Mountain; in southern Maine and sections south of Maine, leaf roll; and in some of the western States spindle tuber and rugose mosaic apparently are the viroses that require primary consid-

eration in control programs. Furthermore, vectors of viroses vary from one locality to another and from season to season in the same locality.

Latent mosaic probably is harbored more generally in important commercial potato varieties in our country than any other virosis. However, as has been pointed out, most of these varieties are masked carriers of this disease so that, unless latent mosaic occurs in combination with other mosaic viruses, it apparently produces only minor effects in these carriers.

With the indication that varieties resistant to latent and mild mosaic can be developed, satisfactory control of at least 2 important virus maladies of potato seems assured.

#### SUMMARY

Because of the variable conditions of infection, the test for resistance to leaf roll and spindle tuber are inconclusive. None of the varieties or seedlings included in the tests were immune from these diseases.

Differences in reaction of Spaulding No. 4 and Green Mountain to streak were found, the symptoms being expressed in milder form in the first-named variety.

Three types of reaction of different potato varieties to streak were observed: (1) Latent or masked carriers, (2) varieties manifesting mild necrosis on the foliage, (3) varieties showing severe dwarfing, curling, and necrosis on tops that usually die before the tubers are formed.

Seedling 41956 was found to be highly resistant to latent mosaic. In field tests S. 42667 also was resistant to this virus but contracted the disease in tuber-graft tests, where it was expressed as top necrosis. Symptomless or masked carriers of this disease include the Green Mountain and other well-known varieties.

The reaction of different potato varieties and seedlings to latent mosaic may be grouped as follows: (1) Highly resistant varieties, (2) varieties resistant in field tests but contracting latent mosaic as top necrosis in tuber grafts, (3) latent or masked carriers.

The reaction of different potato varieties and seedlings to mild mosaic may be grouped as follows: (1) Highly resistant, (2) seedlings that seldom contract mild mosaic in the field but become infected in tuber grafts, (3) seedlings manifesting milder symptoms than Green Mountain, and (4) seedlings contracting mild mosaic as readily as Green Mountain and expressing the symptoms of this disease as clearly as this variety. Seedlings that had previously been selected in breeding-plot tests for possible resistance to mild mosaic when subjected to more severe tests in the disease test plots showed lower percentages of mosaic than the Green Mountain checks grown under the same conditions.

The results of a cross between 2 resistant parents showed transgressive inheritance, some of the seedlings being more resistant than either of the parents, others more susceptible.

More work will have to be done before a complete genetic analysis can be made, but the results so far indicate that there are 2 or more genetic factors involved that are cumulative in effect.

The principal objective of the earlier work, which was to produce varieties of economic importance resistant to mild mosaic, has been accomplished. One of the seedlings, S. 42667, which has been named Katahdin, has shown a high degree of resistance to mild mosaic in all of the tests. This resistance is combined with other characters that are very desirable from the economic standpoint.

Observations on the reaction of potato to mild and latent mosaic have shown that potato varieties that may readily be infected by artificial methods seldom contract these diseases in the field, which indicates that artificial methods of inoculation are not necessarily infallible criteria regarding the reactions of plants under field conditions.

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# A PHYSIOLOGIC FORM OF SEPTORIA TRITICI ON OATS<sup>1</sup>

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## INTRODUCTION

A Septoria leaf-spot disease was found on fall-sown oats (*Avena* sp.) in the Alsea River Valley in western Oregon during the winter months from December, 1930, to March, 1932. It was present both in commercial fields and experimental plots. It was moderately destructive on oats growing in a highly acid (pH 4.8) red soil derived from sandstone and shale. No Septoria leaf spot occurred on varieties of wheat, barley, rye, or spelt growing adjacent to heavily infested oats. A species of Septoria was found on velvet grass (*Holcus lanatus*) in February, 1932. The host and parasite were both prevalent. The fungus on the host and in pure culture is nearly the same as the Septoria on oats. Cross inoculation studies are in progress. The disease also occurred, but with less severity, in the Willamette Valley, adjacent to the Alsea Valley.

Acid soil, open winter weather, and heavy rainfall are predisposing factors in the Septoria leaf spot. The disease may be serious, and, as observed, it seems to be moderately important in small areas on some of the acid soils of western Oregon.

The slender pycnosporos and slow, yeastlike growth on potato-dextrose agar differentiate the fungus from *Septoria avenae* Frank. The latter is the only species of Septoria previously noted on oats in the United States.

## HISTORY

The literature on *Septoria avenae* was reviewed by Weber (10), who showed that this fungus has rather short, cylindrical pycnosporos. He found the ascigerous stage of that fungus, which he named *Leptosphaeria*

<sup>1</sup> Cooperative investigations conducted by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Oregon Agricultural Experiment Station.

<sup>2</sup> Assistant Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The writer is indebted to Dr. G. F. Weber, of the Florida Agricultural Experiment Station, for diagnosing material, and to Dr. S. M. Zeller, of the Oregon Agricultural Experiment Station. He thanks Prof. H. P. Barss, of the Oregon Agricultural Experiment Station, and Dr. A. G. Johnson, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, for constructive criticism of the manuscript. Dr. Johnson obtained literature, photographs of original *erisiccati* and the original Desmazières materials and writings sent by Dr. Foëx from France at the time Dr. Weber was conducting studies under Dr. Johnson's direction at the University of Wisconsin.

*avenaria*, and that it grew moderately fast in culture in contrast to the characteristically slow, yeastlike growth of *S. tritici* Desm. (10, 11).

*Septoria tritici* was described by Desmazières (2) in 1842, as pointed out by Weber (11), on wheat collected by M. Roberge. In 1843, Desmazières (3) described *S. graminum* on dry leaves of grasses (*in foliis siccis graminum*), stating that it was similar to *S. tritici*; in 1847 he (4) reduced *S. tritici* to varietal rank under *S. graminum*, making it *S. graminum* var. *B. tritici*, and he added variety *C. avenae* on oats. He gave the spore length of variety *B. tritici*, but he merely stated that variety *C. avenae* was identical with the type *S. graminum*. Weber (11) concluded that the *Septoria* on wheat leaves in the United States was identical with Desmazières' original *S. tritici* but that it was distinct from *S. graminum*, the latter having obscure pycnidia and very slender pycnosporos less than 1.5  $\mu$  wide. Weber (11) gave *S. tritici* specific rank and suggested that *S. graminum* probably was confined to wild-grass hosts. Weber (10) also stated, after examining type material of *S. graminum* var. *C. avenae*, that this fungus was distinct from *S. avenae*, especially in the more slender spores of the former. Weber (10, 11) did not secure infection on oats with *S. tritici* from wheat nor on wheat with *S. avenae*.

In Europe two species of *Septoria* have been reported on oats, namely, *S. avenae* and *S. graminum*. According to Gram and Thomsen (7), *S. avenae* produces dark spots on the leaves. *Septoria* has been reported on oats from many widely separated localities, most writers referring the fungus to *S. graminum*. Cunningham (1) mentions *S. graminum* on wheat and oats from New Zealand; Marchionatto (8) lists *S. graminum* on oats from the Argentine; Miège (9) refers to *S. graminum* on both wheat and oats from Morocco; and Garbowski (6) lists both *S. graminum* and *S. glumarum* Pass. on dying leaves of oats from Poland.

Diedicke (5) described *Septoria planiuscula* on *Avena planiuscula* (*Mycotheca* March Nr. 1750). The pycnosporos were from 3.5 to 4.5  $\mu$  wide by 30 to 45  $\mu$  long, with seven septa, making *S. planiuscula* clearly distinct from the species herein described.

#### SYMPTOMS

The lesions most frequently develop near the leaf tips but occur on any leaf part, including the sheath. The lesions are circular to elongate. (Fig. 1, A and B.) In many cases the fungus progresses longitudinally between restricting leaf veins and forms long, somewhat rectangular lesions. The lesions are gray with indefinite or yellow margins of various intergradations. (Fig. 1, B.) Most of the yellow or greenish yellow host tissue occurs at the distal end of the lesion, and, in cases where the spot covers most of the width of the leaf, the greenish yellow area also includes all of the leaf beyond the lesion. Obviously, this is attributable to the obstruc-



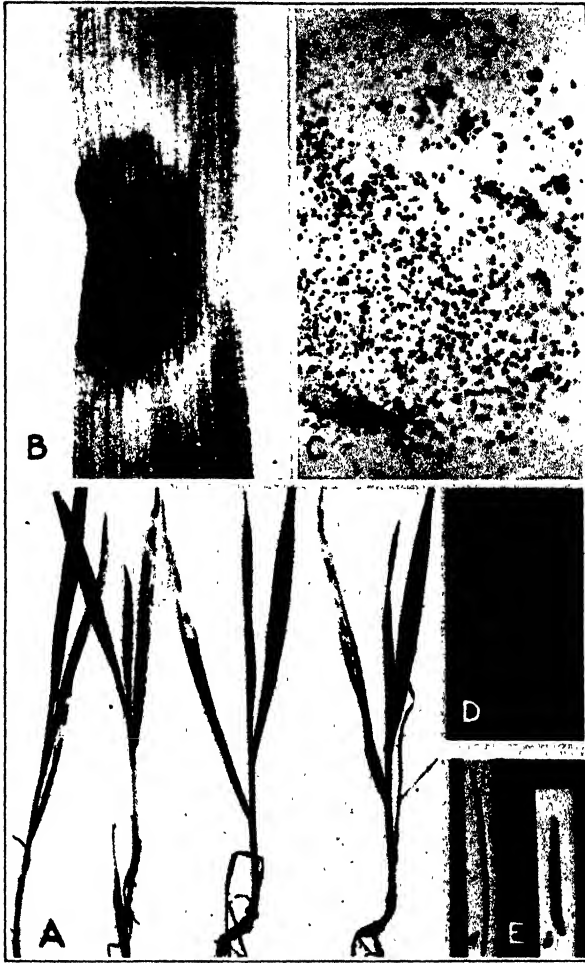


FIG. 1. A. Plants of Red Rustproof oats from Alsea River Valley, Oregon, February, 1931, heavily infected with *Septoria tritici*. B. Enlarged view of a lesion on Red Rustproof oats from Alsea River Valley, Oregon, February, 1931, showing light-colored border, shrunken host tissue, and pycnidia of *S. tritici*.  $\times 4$ . C. Portion of a potato-dextrose agar Petri-dish culture of *S. tritici* isolated from wheat from Willamette Valley, Oregon, and grown 3 weeks at  $15^{\circ}$  C. The plate was inoculated with a spore suspension.  $\times \frac{1}{2}$ . D. Portion of a potato-dextrose agar Petri-dish culture of *S. tritici* isolated from Red Rustproof oats from Alsea River Valley, Oregon, and grown 10 days at  $15^{\circ}$  C. The plate was inoculated with a transfer of mycelium. E. a, pycnosporangium of *S. tritici* from Red Rustproof oats from Alsea River Valley, Oregon, February, 1931.  $\times 423$ . b, Pycnosporangium of *S. avenae* Frank from oats, Puslinch, Ontario, Canada, August 9, 1915. (Fungi Columbiani, No. 4771.)  $\times 423$ .

ting action of the lesion. There may be as many as 10 or more lesions on a leaf blade, but usually there are from 3 to 5. (Fig. 1, A.)

The pycnidia are very prominent on the lesions and occur in parallel rows between the veins of the leaves, with their bases attached or adjacent to the veins. (Fig. 1, B.)

During the winter of 1930-31, *Septoria* leaf-spot lesions were first evident in December, increased in number until early February, and rapidly diminished in number in March as the *Septoria*-infected leaves rotted away and were replaced by new, noninfected growth. The winter of 1930-31 was mild, moderately damp, and followed in early spring by several weeks of exceptionally dry weather. During midwinter, due to the abundant lesions, the growing oats plant had a scorched appearance. Counts made at this time showed that approximately 40 per cent of the leaves had one or more *Septoria* lesions. *Helminthosporium avenae* Eidam also occurred on the leaves.

#### CAUSAL ORGANISM

##### Pure-Culture Studies

The following pure cultures were used in comparative studies on various culture media:

1. *Septoria* isolated from Red Rustproof oats, *Avena byzantina*, in the Alsea Valley, Lincoln County, Oregon, Feb., 1931.
2. *Septoria* isolated from Winter Turf oats, *Avena sativa*, in the Willamette Valley near Jefferson, Oregon, March, 1931.
3. *Septoria tritici* Desm. isolated from winter wheat:
  - a. from fresh host material supplied in the spring of 1931 by E. Gram, Lyngby Experiment Station, Denmark;
  - b. from a collection made in the spring of 1931 at Corvallis, Oregon; and
  - c. from a collection made in the spring of 1931 at High Prairie in eastern Washington.

Comparative studies of these cultures were made chiefly on potato-dextrose agar (pH 5.5) in test tubes and in Petri dishes.

Vegetative cells and spores from young cultures were used for inoculating the agar slants in test tubes. Most of the Petri-dish cultures were made by pouring water dilutions of the pycnospores over the surface of poured plates (Fig. 1, C).

Cultures 1 and 2 were identical. They also were similar to all available cultures of *Septoria tritici* isolated from winter wheat. Colonies from water dilutions of pycnospores on the surface of agar plates formed glistening, flesh-pink spore masses after incubation for several days at 20° C. These colonies consisted mainly of masses of spores in various stages of formation and germination. The hyphae in these colonies were at first fragmentary, and as the colonies grew older, pigmented hyphae with thicker

walls developed and finally covered the entire colonies with a black convoluted stroma or scurf. For a time the flesh-pink spore masses broke through the stroma, but eventually the colonies became masses of carbonaceous mycelia.

The *Septoria* isolated from oats (cultures 1 and 2) grew very slowly (Fig. 1, D) and transfers to test tubes required several months to cover the surface of agar slants incubated at 15° C. In similar cultures grown in Petri dishes the dark macrohyphae and the pigmented hyphae grew beyond the main body of the colony in aster-like rays for a distance of 1 mm. or more. These stellate-margined colonies also were very evident in Petri dishes inoculated by water dilutions of pycnospores.

When the cultures were several weeks or months old they had a tendency to produce white, cottony, aerial mycelia over the earlier-formed stromatic masses. This was common on cultures 1 and 2 from oats but less pronounced on cultures 3a, 3b, and 3c from wheat.

All transitions from pseudopionnotes to well-formed pycnidia were found in pure cultures, the latter being found after transfers on potato-dextrose agar had grown for several weeks at 15° C. In some cultures, pycnidia developed in the agar below the surface, and, being accompanied by only a small amount of vegetative hyphae, were easily seen.

On sterile *Melilotus* stems in test tubes the spores were formed in naked masses, acervuli, and malformed pycnidia.

No ascigerous stage was discovered in cultures isolated from oats grown in the Alsea River Valley. Sterile corn meal, *Melilotus* stems, potato-dextrose agar, and wheat grain were inoculated with pycnospores and with mycelium of the *Septoria* isolated from oats. Cultures were incubated in the ice box and out of doors for several months, but no ascigerous stage was found. A *Leptosphaeria* was occasionally found at the base of old overwintering oats stubble in the field but attempts to isolate it were unsuccessful. The writer questions the connection of the *Leptosphaeria* with the *Septoria* from oats.

In studying the characteristics of spores grown in pure culture it was necessary to deal with fresh transfers grown at fairly uniform temperatures of approximately 15° C. Old colonies or those subjected to widely different conditions produced spores with widely different characteristics, such as vacuolation and distortion of various sorts. Some colonies after being incubated in an ice box for four months bore masses of spores that superficially resembled *Fusarium* conidia.

#### Artificial Inoculations

During the winter of 1930-31, cereals were grown in the greenhouse in pots of soil from the Alsea River Valley and inoculated, by means of atom-

izers, with spore suspensions of culture 1. Winter Turf oats (*Avena sativa*) inoculated and incubated under a bell jar at 50° F. developed typical lesions and pycnidia. Goldcoin wheat similarly treated did not become infected.

In October, 1931, extensive inoculations with culture 1 were made on plants of various cereals and cheat grass 4 to 12 weeks old and incubated in a modified Wisconsin-type spray chamber. The plants were grown in 6-inch pots, 8 plants to a pot; and there were 2 to 4 pots of each variety. The plants were left in the chamber for 16 days. In the first series of inoculations negative results were obtained on all these plants. It was found, however, that the water supplied to the chamber was being inadvertently warmed to 19° C., and after repiping the chamber to permit use of unheated water at temperature of approximately 10° C., infection was obtained on various varieties of oats. The results are given in table 1. Infection is given as positive (+) in the table when one or more plants showed pycnidia in typical lesions. Reisolations were made from each host that became infected. All uninoculated checks remained free from infection. Infection occurred mainly at the tips of leaves and also in other areas on the lower leaves. Data on infection show that the fungus is not a virulent

TABLE 1.—Results of inoculating 6-week-old cereals with pycnosporer suspensions of *Septoria* culture 1 and incubating in a spray chamber for 16 days

Plants inoculated		Results
Genus and species	Crop and variety	
	<i>Oats</i>	
<i>Avena byzantina</i> .....	Red Algerian .....	+
Do. ....	Nortex .....	+
Do. ....	Red Rustproof .....	+
<i>Avena sativa</i> .....	Green Russian .....	+
Do. ....	Iogold .....	+
Do. ....	Silvermine .....	+
Do. ....	Winter Turf .....	+
<i>Avena sativa orientalis</i> .....	White Tartar .....	+
<i>Avena strigosa</i> .....	Sand .....	+
	<i>Barley</i>	
<i>Hordeum vulgare</i> ....	Tennessee Winter .....	-
	<i>Rye</i>	
<i>Secale cereale</i> .....	Common Winter .....	-
	<i>Wheat</i>	
<i>Triticum vulgare</i> .....	Goldcoin .....	-
Do. ....	Huston .....	-
<i>Bromus tectorum</i> .....	Cheat grass .....	-

parasite and that it apparently requires comparatively cool conditions for optimum development.

### Morphology

The morphological characteristics of *Septoria* on oats from the Alsea River Valley, Oregon, are as follows:

The pycnidia are formed in substomatal cavities, their bases being firmly attached to the side of a vein. The pycnidia are amber brown to black and vary somewhat in shape, most of them being globose to subglobose, or ellipsoid with the long axis parallel to the length of the leaf. They are from 40 to 100  $\mu$  wide by 60 to nearly 150  $\mu$  long. The ostioles in mature pycnidia tend to be oval and differ greatly in size, being from 5 to 25  $\mu$  wide by 10 to 36  $\mu$  long, averaging 12  $\mu$  wide by 20  $\mu$  long.

Pycnospores are slender with the ratio of length to width more than 20 to 1. (Fig. 2.) They are narrowly cylindrical, sometimes slightly

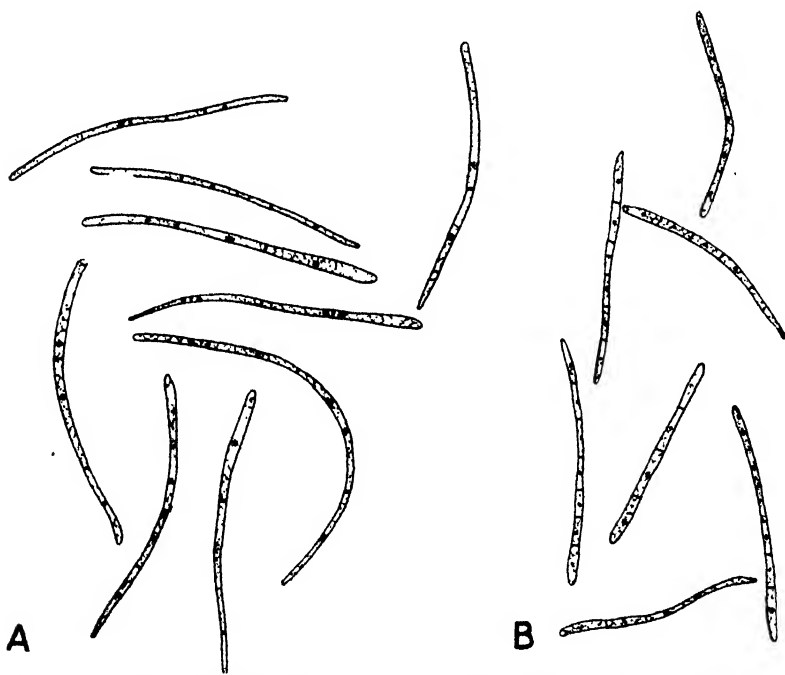


FIG. 2. Pycnospores of *Septoria tritici*. A. From Jenkin wheat, Corvallis, Oregon, March, 1931.  $\times 500$ . B. From oats from Alsea River Valley, Oregon.  $\times 500$ .

swollen at the base, straight to greatly curved, hyaline, and 1–7-septate (usually 2–5-septate). The ends are rounded and the contents homogeneous, with nuclei and nucleoli clearly evident. The spores are from 1.6 to 3.5  $\mu$  wide by 35 to 120  $\mu$  long, averaging 2.3  $\mu$  wide by 68.1  $\mu$  long, 2.1 wide by

67.6  $\mu$  long, and 2.2  $\mu$  by 80  $\mu$  long, respectively, in three collections made during February and March, 1931.

The mycelial development on agar cultures has been discussed. In the host cells, the mycelium is hyaline to yellow or may even be deeply pigmented. It averages approximately 2  $\mu$  in diameter.

### Taxonomy

There are several outstanding differences between *Septoria avenae* Frank and the *Septoria* on oats from western Oregon. The pycnospores of *S. avenae* have a length-breadth ratio of 8–12 to 1 compared with a ratio of usually 20 to 1 or more for the Oregon *Septoria*. (Fig. 1, E.) The two fungi produce entirely different types of colonies on culture media and, finally, there is no ascigerous stage known for the oat *Septoria* from the Alsea River Valley, Oregon, while *S. avenae* is genetically connected with *Leptosphaeria avenaria*.

Type material of *Septoria graminum* var. *C. avenae* Desm., No. 1708 Desmazières, plantes Cryptogames de France Fasc. xxxv. 1847, was examined from material originally sent by Dr. Foëx from France to Dr. A. G. Johnson, Bureau of Plant Industry, U. S. Department of Agriculture. In addition, the corresponding specimen of Desmazières (No. 1708) *exsiccati* in the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, was examined. The first-mentioned material had amber brown pycnidia that were elliptical in cross section and measured 30 to 78  $\mu$  wide by 48 to 108  $\mu$  long (55 by 80  $\mu$ ). The pycnospores ranged from 0.8  $\mu$  wide by 16  $\mu$  long to 1.2  $\mu$  wide by 32.1  $\mu$  long (1.1 by 30  $\mu$ ). Apparently they were nonseptate, but many of these old and brittle spores fractured into three portions as if cross walls might have been forming at the time they were collected. It was suspected that the material sent by Dr. Foëx and seen by Weber (10) had immature spores. The untouched specimen of Desmazières (No. 1708) in the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, showed pycnidia very similar in size and arrangement to those on oats from Oregon. They measured 41 to 82  $\mu$  wide by 70 to 120  $\mu$  long. Approximately 60 per cent of the pycnospores in these well-developed pycnidia were one to four or more septate. These spores measured 0.9 to 2  $\mu$  wide by 28 to 58  $\mu$  long (1.54 by 38.88  $\mu$ ).

These measurements of *Septoria graminum* var. *C. avenae* Desm. are sufficiently close to those of *Septoria* on oats from Oregon to justify including both fungi under the same name. Most of Desmazières' No. 1708 is immature, and this, combined with its great age and shrunken condition, has led to some confusion in the past.

Desmazières' type collection of *Septoria graminum* on wild grass (No. 1328 plantes Cryptogames de France) has dark-brown pycnidia that are nearly circular in cross section and measure 45 to 100  $\mu$  wide by 45 to 103  $\mu$  long, but most of them are uniformly spherical and close to 50  $\mu$  in diameter. The spores of Desmazières' *S. graminum* on grass (No. 1328) from material in the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, and from material in Dr. Johnson's possession, are all nonseptate, so far as it is possible to determine from their present condition. The spores are slightly yellower than the hyaline ones of *S. graminum* var. *C. avenae* and are distinctly shorter and narrower and are frequently less curved than many of the spores of this variety on oats. The differences in spores and pycnidia indicate that the two fungi are distinct. A comprehensive study of many *Septoria* cultures and specimens on grasses will be necessary to determine the exact nature of *S. graminum* Desm. on these grass hosts. It is doubted if it occurs on oats; at least, no specimens have been seen on this host that resemble Desmazières' fungus on wild grass.

The *Septoria* on oats from Oregon, which is identical with *S. graminum* var. *C. avenae* Desm., logically falls under *S. tritici* on account of almost identical morphological and cultural characters. Dr. Weber, who examined one of the collections from the Alsea River Valley, Oregon, stated, in a letter dated February 28, 1931, that he considered that the pycnosporos of the submitted material on oats were within the range of *S. tritici* and quite distinct from *S. avenae* Frank. On account of the sharp division in host range, but only slightly smaller spores (Fig. 2), the form on oats is considered a distinct physiologic form. It must be emphasized that *S. tritici* on wheat has not been known to attack oats nor does the physiological form on oats from Oregon attack wheat.

The writer believes that probably all of the foliicolous *Septoriae* mentioned in literature on oats are either *S. avenae* Frank or *S. tritici* (form on oats).

A technical description of *S. tritici* on oats follows:

*Septoria tritici* Desm., physiologic form on *Avena* (Syn.: *S. graminum* var. *C. avenae* Desm.) not *Leptosphaeria avenaria* Weber (*Septoria avenae* Frank).

#### Description

Lesions on leaves and sheaths gray, irregular or rectangular, partly restricted by veins, indefinite or bordered by greenish yellow areas, which frequently involve the entire distal portion beyond the lesions; pycnidia prominent, scattered in rows parallel to the veins, subepidermal and substomatal with bases appressed to the veins, subglobose to globose, or ellip-

soid with long dimension paralleling main veins, 40 to 100  $\mu$  wide by 60 to 145  $\mu$  long, averaging (February collection) 74 by 95  $\mu$ , walls of pycnidia amber brown to (sometimes) black, smooth, composed of polygonal cells; ostiole oval or sometimes circular 5 to 25  $\mu$  wide by 10 to 36  $\mu$  long (12 by 20  $\mu$ ); pycnosporos exuded in short, pale, flesh-colored cirrhi, cylindrical hyaline, with septa 1 to 7 frequently indistinct, 1.6 to 3.5  $\mu$  wide by 35 to 120  $\mu$  long (2.2 by 70  $\mu$ ), ratio of pycnospore length to width usually greater than 20 to 1.

The hyphae within the host cells are yellow to olive brown and average 2  $\mu$  in diameter.

On *Avena sativa* and *A. byzantina* in western Oregon and on *A. sp.* in France; material was collected near the junction of Alsea River and Five Rivers in Lincoln County, Oregon.

Collections have been deposited in the Oregon State College Herbarium, Corvallis, Oreg., and in the Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

#### SUMMARY

A locally prevalent *Septoria* leaf spot was found on cultivated oats growing in an acid soil in the Alsea River Valley in the coastal region of western Oregon. The disease was most destructive during the months of December, 1930, to February, 1932, inclusive.

Because of its slender spores and slow, yeastlike growth in culture, the fungus is distinct from *Septoria avenae* Frank and is morphologically very similar to *S. tritici* Desm. It has been designated as a physiologic form of *S. tritici* Desm. A full technical description is given.

The form on oats is identical with *S. graminum* var. *C. avenae* Desm. and the latter is distinct from *S. graminum* Desm. on grass. The form on oats did not attack wheat and the form or forms on wheat so far have not been found on oats.

It is suggested that all the reported cases of *Septoria* on leaves of oats probably are either this physiologic form of *S. tritici* on oats or *S. avenae* Frank.

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# THE EFFECT OF SULPHUR FUNGICIDES, APPLIED DURING THE BLOOM, ON THE SET OF APPLE FRUITS

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## INTRODUCTION

These studies supplement a previous publication<sup>1</sup> in which the literature was reviewed. On the basis of the data then presented it seemed evident that sulphur, if in contact with the pollen, inhibited its growth on agar media; that sulphur applied to the stigmas before or shortly after pollination reduced the set of fruit; and that sulphur, applied 24 to 48 hours after pollination, had little effect on the set. Since that time, additional work in the orchards at Ithaca, in the Champlain Valley, and in western New York has been carried on to obtain more data bearing on the problem. The points given particular consideration were, 1st, the effect of lime-sulphur spray applied during full bloom as compared with sulphur dust; 2nd, the length of time after pollination before sulphur dust or lime-sulphur spray could be applied without reducing the set; 3rd, the effect of the application of these fungicides to whole trees under orchard conditions; and 4th, the length of time necessary for the pollen tube to traverse the style of the apple blossom under different temperature conditions, as tested by a method other than sulphur application. It is felt that publication of the data at this time is appropriate because of renewed interest in spraying or dusting during bloom in the control of apple scab and other diseases.

## EXPERIMENTS

In the first series of experiments, 6 Northern Spy trees 20 years of age, in a cultivated orchard, were chosen for comparable vigor. On each of these trees 50 vigorous spurs were selected, the blossoms thinned to 2 per spur, and the spurs enclosed in glassine bags. Four different treatments were given to each tree, there being 10 to 12 bags including 10 to 22 spurs in each treatment. All blossoms were pollinated with Rome pollen on the same day. Dusting sulphur was applied to the stigmas at the intervals indicated in table 1. Temperature was favorable for pollen-tube growth throughout, the maximum being 81°, 88°, 82°, and 82° F. on successive days.

It is to be noted in this series that the average per cent set increases directly with the interval between pollination and application of sulphur.

<sup>1</sup> MacDaniels, L. H., and J. R. Furr. The effect of dusting-sulfur upon the germination of the pollen and the set of fruit of the apple. N. Y. (Cornell) Agr. Expt. Sta. Bul. 499. 1930.

TABLE 1.—*Set of fruit as affected by the application of dusting sulphur to stigmas at time of pollination and at intervals after pollination. Northern Spy, 1932. Set after June drop in per cent of spurs treated*

Interval hours	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Tree 6	Ave.
0	13	45	47	26	33	21	31
24	25	56	31	31	25	36	34
72	76	66	46	56	68	53	61
Check	72	70	89	63	80	82	76

The most significant difference is between the 24- and 72-hour interval. In all cases the set on the spurs receiving sulphur immediately or within 24 hours after pollination was much less than the pollinated check and in all but one, less than that on the spurs that received sulphur after the 72 hour interval. Table 2 gives the results obtained on 5 McIntosh trees chosen in the same way as the Northern Spy trees referred to in table 1. Here, with one exception, the application of sulphur either before or coincident with pollination reduced the set as compared with the checks.

TABLE 2.—*Set of fruit as affected by the application of dusting sulphur to the stigmas at different times with relation to pollination. McIntosh, Spring, 1932. Set after June drop in per cent of spurs treated*

Treatment	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Ave.
Sulphur and pollen applied at the same time	17	17	43	21	33	26
Sulphur applied 24 hours after pollen	47	20	44	23	17	30
Pollen applied 24 hours after sulphur	37	5	29	18	22	22
Pollen only applied	39	20	64	30	53	41

In a third experiment, unit branches about an inch in diameter were covered with 3 × 6 ft. cheesecloth bags. Pollen was applied with a small brush. The sulphur was blown on with a duster. The results are summarized in table 3. Here again, the application of dust before the pollen gave the greatest reduction in set. In another trial with McIntosh in 1930, 2 hand-pollinated bagged unit branches set fruit on 38 to 43 per cent of

TABLE 3.—*Set of fruit on bagged hand-pollinated unit branches as affected by dusting with sulphur. Variety McIntosh. Spring, 1932*

Treatment	Tree	No. blossom spurs	Per cent growing points blossom	No. spurs with fruit Sept.	No. fruits Sept.	Per cent blossom spurs with fruit Sept.
Pol. May 24 ....	1	87	75	27	28	31
Dust May 24 ....	2	81	57	41	44	50
Average ....						40.5
Pol. May 24 ....	1a	87	52	46	63	53
	1b	120	85	72	88	60
Dust May 25 ....	2	72	50	43	51	60
Average ....						57.6
Pol. May 25 ....	1	45	33	14	16	31
	2a	84	71	27	32	32
Dust May 24 ....	2b	131	78	18	18	14
Average .....						25.6
Pol. May 24 ....	1	56	35	32	45	57
No dust .....	2	113	63	45	50	40
Average .....						48.5

their blossoming spurs as compared with 20 per cent on a comparable branch dusted immediately following pollination.

The results of the most extensive experiments with unit branches are given in table 4. In this series the unit branches were chosen for their uniformity, on 13 different trees, and covered with 3×6 foot cheesecloth bags, as in the preceding series. The pollen was applied by hand, the dust with a crank-type duster, and the lime-sulphur spray (1-40) with a 3-gallon hand sprayer. On the bagged branches either lime-sulphur spray or dust applied immediately following pollination greatly reduced the set of fruit. Application after 24 hours reduced the set considerably, but differences at longer intervals were not significant. On the uncovered branches the application of lime-sulphur spray reduced the set 20 per cent as compared with the checks.

In another series of experiments, groups of 8-year-old trees, chosen for uniformity and adjacent position, were dusted and sprayed at various times as indicated in table 5. The trees were somewhat small for their age and were growing in alfalfa sod. They were, however, in fair vigor. The small volume of the crop for trees of this age is in large part due to the small size of the apples, caused by late season drought.

Although the comparison between whole trees at this early bearing age is likely to be unsatisfactory because of the natural variation in the amount

TABLE 4.—*Set of fruit on unit branches as affected by the application of lime-sulphur spray and sulphur dust during full bloom. McIntosh, Spring, 1932*

Treatment	No. branches	Bagged (B) or open (O)	Per cent blossom spurs with fruit June	No. fruits per 100 blossom spurs June	Per cent blossom spurs with fruit Sept.
Check .....	13	O	64	93	25
L. S. ....	5	O	40	48	20
Pollen only .....	13	B	71	115	37
P. + L. S. at once ....	5	B	30	35	13
P. + L. S. 24 hours ..	1	B	65	91	28
P. + L. S. 27-29 hrs. ..	9	B	54	79	28
P. + L. S. 48 hours (in one case 53 hrs.) .	5	B	68	107	30
Pollen only .....	13	B	71	115	37
P. + S. dust at once .....	2	B	28	34	13
P. + dust 24 hrs. ....	1	B	56	81	26
P. + S. dust 27-29 hrs.	9	B	68	108	38
P. + S. dust 48 hrs. ....	3	B	60	100	29

of crop borne, certain things stand out. In all 5 groups, lime sulphur applied on May 26 greatly reduced the set as compared with the adjacent checks. Trees dusted both on May 26 and May 27 also showed a much poorer set than the adjacent checks. The application of lime sulphur or dust on May 27, however, showed no significant reduction of set. It should be pointed out in this connection that variation in results on whole trees in an experiment of this sort is very likely to occur because of the difference in the stage of bloom of different trees on the same day. As indicated be-

TABLE 5.—*Set of fruit on whole trees as affected by spraying with lime-sulphur and dusting with sulphur during full bloom. Eight-year-old McIntosh. Spring, 1932. (Yield in bushels per tree)*

Treatment		Group 1	Group 2	Group 3	Group 4	Group 5
May 26	May 27					
Lime sulphur		.3	.4	1.3	1.7	1.7
	Lime sulphur	.8	1.5			
Dust	Dust	.5	.3			
	Dust	1.0	.8	1.5	2.0	
Dust				2.0	2.5	
None	None	.9	.6	1.8	3.0	2.2

fore, the stage of bloom when sulphur is applied is very important, as fungicides applied in the early stages of bloom reduce the set much more than those applied late after pollination has occurred.

Since the effect of sulphur in reducing the set of fruit depends on the time of application with reference to pollination, it is of value to know the length of time required for the pollen-tube to grow down through the styles and accomplish fertilization. To get data on this point, a series of experiments was laid out, adapting the method used by Sandsten.<sup>2</sup> Trees were chosen for their comparable vigor and 50 vigorous spurs selected on each tree. Each spur was thinned to 2 blossoms and covered with a glassine bag. All the blossoms were pollinated on the same day and the styles were cut off at the base with a pair of sharp scissors, at the indicated intervals after pollination. The data presented in table 6 show that McIntosh set no

TABLE 6.—Set of fruit as affected by cutting off stigmas at different intervals of time after pollination

McIntosh, 1930

Interval hours	Set on 10 spurs				
	Tree 1	Tree 2	Tree 3	Tree 4	
19	0	0	0	0	
24	0	0	0	0	
30	0	0	0	0	
48	0	2	0	0	
54	3	2	1	0	

Northern Spy, 1930

Interval hours	Set on 10 spurs					
	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Tree 6
25	0	0	0	0	0	0
30	0	0	0	0	0	0
50	0	0	0	0	0	0
66	0	1	0	0	0	0
75	6	1	8	0	0	0
Check	8	6	7	4	5	3

fruits until after an interval of 48 hours, and in 2 out of 3 cases only after 54 hours. With Northern Spy, the first fruit set after 66 hours. Two trees set well after 75 hours, and on 3 trees no fruits set that had the stigmas removed. Difference in the length of time in the case of McIntosh as com-

<sup>2</sup> Sandsten, E. P. Some conditions which influence the germination and fertility of pollen. Wis. Agr. Expt. Sta. Res. Bul. 4: 149-172. 1909.

pared with Northern Spy is correlated with the maximum temperatures, which, in the case of McIntosh, were 74, 74, and 77 degrees on 3 successive days; whereas in the case of the Northern Spy series, the maximum temperatures were respectively 65, 59, 62, and 68 degrees. This series is not altogether satisfactory because of the light set obtained. It is thought this might be explained in part by the fact that the bags were not put back on the blossoms after the stigmas were cut, and hence the bases of the styles dried out sufficiently to damage the flowers. This is borne out somewhat by the series on Northern Spy in 1931, as indicated in table 7. Here the bags were put back over the flowers after cutting the stigmas and a good set was obtained on all spurs with the stigmas cut after an interval of 48 hours. Maximum temperatures in this series were 74, 84 and 83 degrees on successive days.

TABLE 7.—*Set of fruit as affected by cutting off stigmas at different intervals of time after pollination. Northern Spy. Spring, 1931*

Tree	Interval hours	Number bags	Number spurs	Number spurs with fruit	Number fruits	Per cent spurs with fruit
1	22	10	15	0	0	0
	49	15	17	10	17	59
	73	10	12	3	4	25
	Check	10	25	19	30	76
2	22	10	14	0	0	0
	48	15	21	11	12	52
	72	10	12	6	7	50
	Check	10	11	6	8	54

#### DISCUSSION

The data given above in general confirm the conclusions given in the previous publication.<sup>3</sup> The application of sulphur to the stigmas of flowers on individual spurs did not reduce the set in these experiments so markedly as in the previous experiment probably because of a difference in method. In the previously published work the spurs were thinned to 2 blossoms and sulphur applied to only one of the blossoms on the spur. Under these conditions, practically all of the blossoms treated with sulphur failed to set as they were apparently at a disadvantage in competing with the untreated flower on the same spur.

Considering all data, it seems without question that sulphur applied as dust or lime-sulphur spray, either before pollination takes place or shortly after, reduces the set of fruit. In view of the inhibitory effect of sulphur on pollen germination on agar media, it is perhaps surprising that the set is

<sup>3</sup> See footnote 1.

not reduced more than it is. A possible explanation is that there may be a sufficient number of pollen grains on the stigmas that are not in contact with the sulphur to accomplish fertilization, in spite of the effect of the sulphur upon a large proportion of the pollen grains present. Also, under normal orchard conditions there are usually either enough blossoms that have already been pollinated for some time, and hence not susceptible to sulphur injury, or sufficient unopened blossoms that do not receive sulphur on the stigmas, and hence can be pollinated later, to give a satisfactory crop. There are some indications that application of lime-sulphur spray reduces the set more than an application of sulphur dust. In these experiments spray or dust applied early in the blooming season reduced the set more than the same application toward the end of the blooming season.

From the orchardists' standpoint a moderate reduction in set on varieties normally requiring thinning is sometimes advantageous. It should be pointed out that the varieties used in these tests are self sterile and frequently fail to set because of a lack of pollination. The use of varieties like Wealthy and Baldwin, which usually set when they bloom, might have given different results. It is certain that in many orchards in many seasons trees could be dusted during bloom without serious reduction in the volume of fruit harvested. On the other hand either spraying or dusting in bloom in seasons that are cold or otherwise unfavorable for pollination may reduce the set below a commercial crop.

The data obtained by removing the styles from blossoms at intervals after pollination indicate that under favorable temperature conditions pollen tubes may traverse styles of apple blossoms in 48 hours or less. This coincides in general with the interval after pollination at which sulphur fungicides may be applied without reducing the set.

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# DIPLODIA EAR ROT IN INBRED AND HYBRID STRAINS OF SWEET CORN<sup>1</sup>

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## INTRODUCTION

During the field testing of a large number of inbred and hybrid strains of sweet corn in 1930, opportunity occurred to secure data on the relative susceptibility of the different strains to the dry rot of corn, following natural infections by *Diplodia zeae* (Schw.) Lév. Data were taken also from a similar plot of dent corn planted in part of the same field and subjected to the same environmental conditions. It was thus possible to obtain a comparison of the relative amounts of ear infection in these 2 classes of corn.

The dry rot of corn, caused by *Diplodia zeae*, is now recognized as the most serious corn ear rot in the Central States. This fungus causes the most direct and most obvious damage as a mold or rot on the ear, where it seriously affects the quality of the seed. It may attack also the mature plant at various places on the stalk and thereby cause premature ripening of the ear.

This disease occurs in most of the principal corn-growing sections, especially from Nebraska eastward, but has rarely been found in the Pacific Northwest (3). Durrell (2) reported that the losses in Iowa in dent corn in 1921 and 1922 ranged from 3 to 15 per cent of the ears at harvest and caused an average damage to seed corn of 11 per cent. The annual loss in yield of corn in Illinois due to *Diplodia* seed infection is estimated at 2 per cent of the entire crop by Koehler and Holbert (5).

Losses due to dry rot may occur at 3 stages in the growth of the corn plant, *i.e.*, the seedling, the mature plant, and the ear. Heavily infected seed will ordinarily fail to germinate or, at best, will produce a weak plumule, which is quickly rotted off. Slightly infected seed may germinate but the resulting seedling will be rotted off or the growth will be so retarded that the plants will not develop normally. A high percentage of such stunted plants will produce nubbins, or partially filled ears, or may fail to develop ear shoots. Stalk infections, which frequently result in premature ripening of the plant, are produced from spores coming from pycnidia that

<sup>1</sup> Cooperative investigations, Purdue University Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

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have overwintered on old corn stalks. Burrill and Barrett (1) demonstrated that the spores may be carried considerable distances by the wind. Infections developing on the ear from spores falling into the space between the husks and the ear, under proper temperature and moisture conditions, may spread over the kernels as a mycelial mat and render the ear useless either as seed or food.

Holbert, Burlison, *et al.* (4), have indicated that "differences in resistance and susceptibility to the disease exist in both open pollinated and inbred strains" of corn.

#### MATERIALS AND METHODS

The results presented in this paper are based on counts of the number of infected ears found in the various inbred lines and hybrids of sweet corn. Very little *Diplodia* is evident when the sweet corn is in canning condition. Surveys made annually for the last 10 years in Indiana and surrounding States have failed to reveal more than an occasional isolated case of ear infection in roasting ears. On the other hand, the fungus attains its best development when seed corn is being harvested.

In 1930 a number of the better inbred strains and their first-generation hybrids were planted in a field that had been in continuous corn culture over a period of 12 years. During preceding years, the stalks had been left standing on this field until spring, when they were ploughed under as thoroughly as possible with the ordinary plow. However, a rather heavy litter of old stalks, profusely covered with pycnidia of *Diplodia zeae*, was present on the surface of the soil. This inoculum furnished a continuous natural source of spores throughout the season.

In this particular growing season the September and October temperatures were high (September average 65.7° F., 2.1° F. above normal, and October, 53.5° F., 0.81° F. below normal.) Durrell (2) has shown that the optimum temperature for the development of *Diplodia zeae* is between 82° and 86° F., but the fungus develops readily at temperatures above 68° F. Durrell also has shown that "heavy rainfall at the end of the growing period very materially favors the development of *Diplodia*." The rainfall in September was 2.76 inches and in October 0.89 inch, which was sufficient to support the growth of the fungus.

#### RESULTS

##### Comparison of Occurrence of *Diplodia zeae* on Sweet Corn and Dent Corn

The various strains of sweet corn were all harvested on October 14. The ears from each row, laid at the end of the row, were then classified into 2 groups: (1) clean, bright, and apparently disease-free; and (2) those that

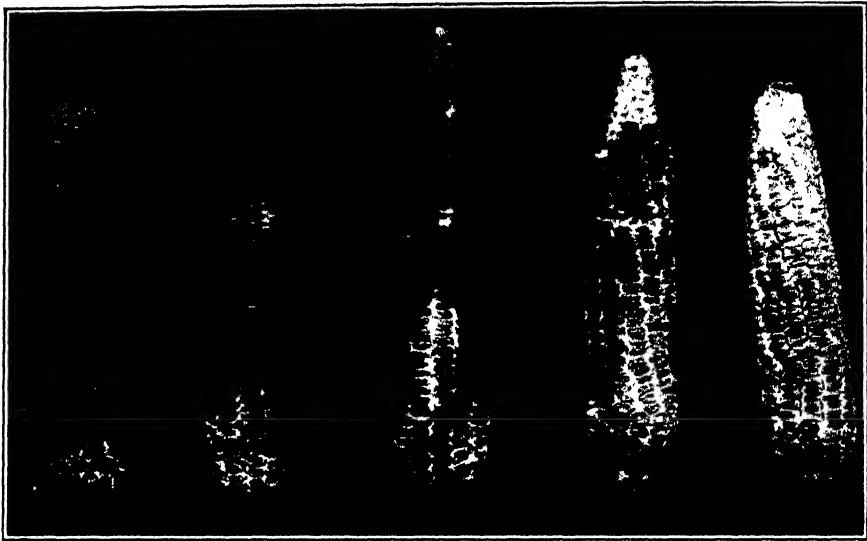


FIG. 1. Ears of Golden Bantam sweet corn showing different degrees of infection with *Diplodia zeae*.

showed any infection with *Diplodia*. The appearance of sweet corn ears carrying different amounts of infection is shown in figure 1. The ears were then counted and the results are summarized in table 1. A similar procedure was followed for the strains of dent corn, which were harvested 2 weeks later.

Results presented in table 1 indicate a difference of 5 per cent in the amount of natural ear infection in the sweet and dent corns. In the dent-corn group there is an apparent correlation between earliness of maturity and the amount of infection by *Diplodia*. That is, the early maturing strains of dent corn had relatively high percentages of infected ears and the late types showed low percentages of ear rot. None of the sweet-corn varieties had so high a percentage of infected ears as did the early Yellow Dent strains that matured along with the sweet-corn strains and were subjected to the same exposure to infection.

It has been generally believed that sweet corn is more susceptible to disease than the dent corns. Reddy, Holbert, and Erwin (6) make the following statement: "Sweet corn and dent corn have many diseases in common but in practically all instances the effects are more severe on sweet corn. The varieties of sweet corn of high quality in respect to sugar content are especially susceptible."

Here, where equal opportunity was afforded for these strains to become infected and where so many widely different strains were present, varying from the early maturing strains of Golden Bantam to the latest Narrow-

TABLE 1.—*Comparison of ear infection with Diplodia zeae in inbred and hybrid strains of sweet and dent corn<sup>a</sup> at Lafayette, Indiana, 1930*

Kind of corn and variety	Season	Inbreds or hybrids	Number of strains	Average per- centage of ears infected
<i>Sweet corn</i>				
Golden Bantam	Early	Inbreds	69	13.0
		Hybrids	21	5.0
Country Gentleman	Midseason	Inbreds	70	9.0
		Hybrids	64	23.9
Early Evergreen	Midseason	Inbreds	11	28.0
Stowell Evergreen	Late	Inbreds	13	12.0
		Hybrid	1	0
Narrow-Grain Evergreen	Late	Inbreds	10	22.0
Total			259	
Weighted average				14.8
<i>Dent corn</i>				
Yellow Dent	Early	Inbreds	13	33.0
Reid Yellow Dent	Medium	Inbreds	50	14.6
		Hybrids	37	5.0
Reid and Johnson County White	Late	Inbreds	72	1.8
Total			135	
Weighted average				9.5

<sup>a</sup> Data on dent corn kindly supplied by R. R. St. John, Associate Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

Grain Evergreens in sweet corn and from the early dent strains, such as Golden Glow, to the late strains, such as Johnson County White, there were apparently no significant differences in the percentages of ear infection by *Diplodia zeae* in the 2 classes of corn.

#### Occurrence of *Diplodia zeae* in Different Strains of Sweet Corn

A more detailed study was made of the occurrence of *Diplodia zeae* among the different strains of sweet corn. A summary of the range of infection among 225 different strains is presented in figure 2. In this figure the strains were grouped into classes differing by 10 per cent; and the heights of the columns indicate the number of strains in each class.

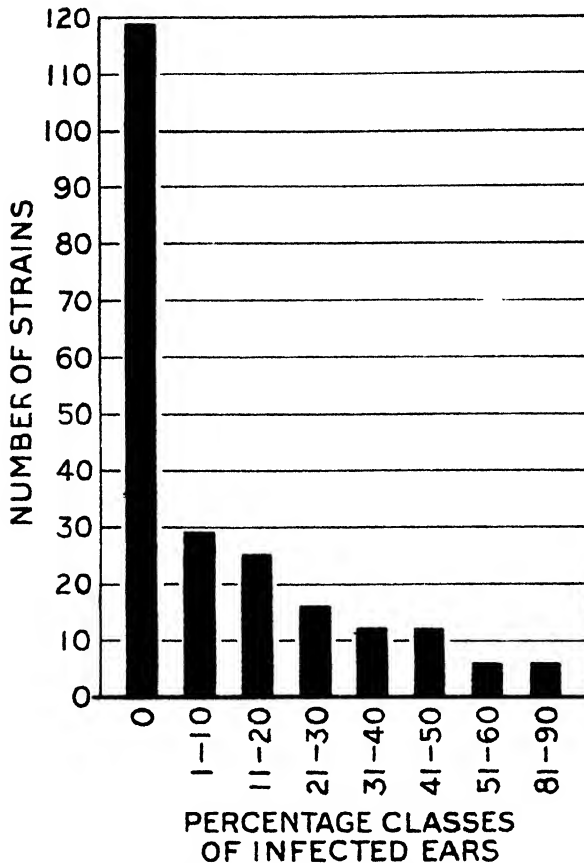


FIG. 2. Graph showing ear infection with *Diplodia zeae* in strains of sweet corn.

According to Koehler and Holbert (5) differences in resistance and susceptibility to *Diplodia* exist in both open-pollinated and inbred strains of dent corn.

The results presented in figure 2 show that a few of the strains, approximately 10 per cent, developed more than 30 per cent infected ears. Apparently, strains of sweet corn resistant to *Diplodia zeae* may be secured by careful selection among selfed lines under conditions of heavy natural exposure to infection by this fungus. The differences among strains developing side by side and maturing at the same time are significant enough to demonstrate the value of such a method of control.

#### Comparison of Ear Infection by *Diplodia zeae* in Inbred Strains of Sweet Corn and in Their First-Generation Crosses

Inbred strains of sweet corn and their first-generation crosses were planted in adjacent rows in a number of these trials. Consequently, it was

possible to make direct comparisons of the percentages of infection in the various selfed lines and in their hybrid recombinations. The results of these comparisons by varieties are summarized in table 2.

TABLE 2.—*Ear infection with Diplodia zeae in inbred strains of sweet corn and in their first-generation crosses*

Variety	Number of strains	Average percentage of ears infected in		F <sub>1</sub> hybrids
		Pistillate parent	Staminate parent	
Golden Bantam . . . .	20	13.0	15.3	5.0
Country Gentleman	64	12.3	11.9	23.9
Stowell Evergreen . .	1	35.0	40.0	0

Less infection occurred in crosses of Golden Bantam and Stowell Evergreen varieties, respectively, than in the parent inbred strains of these crosses. However, in the Country Gentleman strains, there was a decided increase in the percentages of infection in the hybrids, amounting, essentially, to a summation of the percentages of the 2 parents.

#### Influence of Method of Selection on Occurrence of *Diplodia zeae* in Sweet Corn

Certain inbred lines used in the plot had been selected solely on the basis of canning value during the first 5 years of their inbreeding, while others had also been selected on the basis of disease resistance. A comparison of the percentage of *Diplodia*-infected ears in these 2 groups in each of 4 varieties is presented in table 3.

TABLE 3.—*Percentages of ear infection by Diplodia zeae in inbred strains of sweet corn. Group I, selected on the basis of canning value only, and Group II, selected for disease resistance and quality*

Variety	Number of strains in		Average percentage of infected ears in	
	Group I	Group II	Group I	Group II
Golden Bantam . . .	16	53	8.3	7.3
Country Gentleman	52	18	18.5	3.3
Narrow-Grain Evergreen	15	28	6.5	3.0
Stowell Evergreen . .	5	8	12.4	8.7

Under conditions where *Diplodia zeae* annually causes losses in sweet corn, it is apparent from the results presented in table 3 that losses from

Diplodia ear rots can be partly controlled by vigorous selection for resistance. The data show the greatest response to such selection in strains of Country Gentleman, being reduced from 18.5 per cent to 3.5 per cent and the least response in those of Golden Bantam, in which they were reduced from 8.3 per cent to 7.5 per cent. The latter variety usually is considered especially susceptible to disease but, under the conditions of this experiment, showed considerable resistance to infection by *D. zeae*.

#### SUMMARY

Trials of a large number of strains of dent and sweet corn under conditions of heavy natural infection with *Diplodia zeae* indicate that there is little difference in the average percentages of naturally infected ears in dent corn and in sweet corn.

In these comparisons there was little evidence of any correlation in the percentage of Diplodia-infected ears in the inbred strains and in the first-generation crosses between them.

The results indicate that breeding and selection for resistance will tend to reduce losses from *Diplodia zeae*, particularly in certain varieties of sweet corn.

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# PRODUCTION OF CABBAGE SEED FREE FROM PHOMA LINGAM AND BACTERIUM CAMPESTRE<sup>1</sup>

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Epidemics of cabbage black leg (*Phoma lingam* (Tode) Desm.) and black rot (*Bacterium campestre* (Pam.) E. F. S.) are commonly initiated from inoculum carried with the seed. The writer<sup>2</sup> has previously shown the importance of rainfall in the dissemination of the black-leg organism in the seed bed from foci arising from infected seeds. It was pointed out that the Skagit Valley, Washington, seed-growing region in the vicinity of Puget Sound appeared to be free from this disease. Moreover, experimental plantings of infected seed in that area resulted in little or no development of black leg in seed beds and in seed fields planted therefrom.<sup>2,3,4</sup> This was attributed to the low rainfall of that section during a period of some 3 months after cabbage seed is sown.

Since 1921 many seed lots grown in the Skagit Valley area have been studied under Wisconsin conditions. Although parallel plantings from infected seed showed abundant development of the two diseases, neither black leg nor black rot has been definitely shown to be carried with seed grown in the Skagit Valley. This result prevailed throughout, although certain of the lots tested were known to have been grown from black-leg-infected Wisconsin-grown seed stock.

This paper is a report on certain studies of a similar nature on black rot, and some observations on black leg that confirm previous findings.

## RELATION OF RAINFALL TO DISSEMINATION OF BLACK ROT

Black-rot-free seed was demonstrated by immersing it in a suspension of black-rot bacteria. The seed was sown out-of-doors at Madison, Wisconsin, on May 17, 1929, in 2 adjacent rows 12 inches apart. On either side of these, 3 rows of clean seed were sown at the same interval. One-half of each row was covered during rainy periods and the plants were irrigated by running water between the rows with care to avoid splashing. On July 1, when the

<sup>1</sup> Cooperative investigations between the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Dept. Agr. and the Department of Plant Pathology, University of Wisconsin.

<sup>2</sup> Walker, J. C. Seed treatment and rainfall in relation to the control of cabbage black-leg. U. S. Dept. Agr. Bul. 1029. 1922.

<sup>3</sup> ———. Occurrence and control of black leg of cabbage. (Abst.) Phytopath. 10: 64. 1920.

<sup>4</sup> ——— and W. B. Tisdale. Further notes on the occurrence of cabbage black leg. (Abst.) Phytopath. 12: 43. 1922.



plants had reached the transplanting stage, they were removed and examined for black-rot symptoms. The data recorded in table 1 show clearly

TABLE 1.—*Relation of rainfall to dissemination of Bacterium campestris in a seed bed. Madison, Wisconsin, 1929*

Row number	Seed inoculated or clean	Exposed to normal rainfall		Protected from rain	
		Total plants	Diseased	Total plants	Diseased
		<i>Number</i>	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>
1	clean	177	1.7	105	0.0
2	do	250	2.0	175	0.6
3	do	183	14.8	134	1.5
4	inoc.	203	81.8	188	52.1
5	do	<sup>a</sup>		177	63.3
6	clean	101	54.5	157	0
7	do	121	37.2	162	0
8	do	107	7.5	67	0

<sup>a</sup> Plants were not removed at the time final notes were taken, since they were needed later for another experiment; the disease was approximately equal in extent to that in row 4.

that the organism spread readily from the inoculated rows to those adjoining in the unprotected portion of the plot. When the factor of spattering rain was eliminated there was very little spread from inoculated rows. It is evident that spattering rain, as in the case of black leg, is an important means of dissemination of the black-rot organism in the seed bed.

In the Skagit Valley area seed is sown, depending upon the variety, from May 15 to June 15. In general, the rainfall in this area is very light from the middle of May to about September 1. The fact that the dissemination of the black rot organism as well as that of black leg is greatly reduced in the absence of rain suggested an explanation of the general lack of the former in the western Washington cabbage-seed fields. Comparative plantings were made at Mount Vernon, Washington, and at Madison, Wisconsin, in 1931 and 1932.

#### COMPARATIVE DEVELOPMENT OF BLACK ROT IN WISCONSIN AND WESTERN WASHINGTON

Inoculated seed was sown at Madison on May 13, 1931, and a few days later at Mount Vernon. On July 6, 28 per cent of the plants at Madison showed black-rot symptoms. On July 13, the seed bed at Mount Vernon was examined. No external signs of black rot were found. One plant in about 200 showed slight vascular discoloration when the stems were cross-sectioned.

In 1932 a lot of seed naturally infected with *Phoma lingam* was used. This seed was inoculated with the black-rot organism. Part of it was planted at Madison on May 13 and the remainder at Mount Vernon a few days later. On July 27, 54 per cent of the plants at Madison showed signs of black rot and 18 per cent showed black leg. On July 1, about 1500 plants from the Mount Vernon bed were transplanted. On July 18 the plants remaining in the seed bed and those in the field were examined. In the seed bed only one plant out of several hundred showed unmistakable signs of black rot. In the field no signs of the disease were to be found. On August 31 the field planting was again examined. About 4 per cent of the plants showed systemic lesions of black rot. At no time was there any evidence of black leg found either in the seed bed or in the transplanted field.

From these observations of 2 years it is evident that development of black rot in the seed bed is negligible in the Skagit Valley area. It should be emphasized that the seed in both of these experimental plantings carried a much heavier black-rot inoculum than is ordinarily the case with naturally infected seed.

#### DISCUSSION AND SUMMARY

Black leg and black rot of cabbage have caused repeated losses due to the introduction of their pathogens with the seed. Ordinarily, the course of development is a progressive one from scattered local centers in the seed bed. This spread is dependent largely upon spattering or wind-blown rain drops. A decade or more of observations of the cabbage seed crop in the Skagit Valley seed-growing area and of the crops grown from such seed crops has substantiated the belief that cabbage seed grown in this area is generally free from the pathogens of the above-named diseases.

This conclusion has been further strengthened by experimental plantings of infested seed in this area. It is believed that the very low precipitation during a 3-month period following the sowing of seed is the most important factor in checking the spread of the parasites.

In recent years a large proportion of commercial cabbage seed production in the United States has been transferred to this area. This change has been stimulated in part by the increasing evidence of its freedom from important seed-borne parasites.

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MADISON, WISCONSIN

## PHYTOPATHOLOGICAL NOTES

*Giant Galls Caused by the Root-knot Nematode.*—The largest nematode galls ever recorded were observed recently on some ornamental *Thunbergias* from Florida. Figure 1, B, gives a view of such a gall located at the base of a *Thunbergia laurifolia* Lindl. vine, growing on the grounds of a hotel in Palm Beach in 1925. Similar large galls occurred at the bases of a number of other *Thunbergia* vines on these grounds. Figure 1, A, represents two pieces from a similar gall on *Thunbergia grandiflora* Roxb., collected in 1933 on a property near Babson Park, where several of these vines have developed these galls within the last few years. Similar galls on this host have been seen repeatedly in peninsular Florida, and Miss Nellie A. Brown kindly supplied a picture of one collected in 1921 in Cienfuegas, Cuba. The gall-like masses of tissue, caused by the root-knot nematode *Heterodera marioni* (Cornu 1879) Goodey, 1932, often reach a diameter of 18 inches to 2 feet, and may attain this degree of development within a period of a few months to a year. Neither of the *Thunbergias* seems to suffer much from these galls, except perhaps by a reduction in the number of flowers. Older galls soon decay and crumble. In a fresh condition the gall tissue is extremely tough, consisting mainly of lignified fibers. The gall surface is very rugose and of blackish color, whereas the interior has a waxy, yellowish-white appearance. A histological study has not yet been successful because of the extreme hardness of the gall tissues.

Concerning the location of these galls on the host plant, it is remarkable that they do not occur on the root but on the basal stem. They can best be compared with galls produced by the same nema on tubers, corms, rhizomes, and other subterranean stems, with one striking difference, however, that in this case they are formed above ground, as is evident from Fig. 1, B.

Root-knot galls on the basal portions of stems above ground have also been observed on two other plants, and in both instances the whole appearance and character of these galls was similar to that of those on the *Thunbergias*. The surface is blackish and very rugose, the size also large. Figure 1, E, represents one of this type on the rhubarb, *Rheum rhaponticum*,<sup>1</sup> and Fig. 1, C, on a begonia, *Begonia* sp.<sup>2</sup> From these cases it may be concluded that such giant, extremely rugose, crown-gall-like root-knot galls are probably always basal stem galls. Why it is this region of a plant that responds to an infestation by this nema with such noticeable cancerous

<sup>1</sup> Through the courtesy of Miss Nellie A. Brown, of U. S. Bureau of Plant Industry.

<sup>2</sup> From B. G. Sifton of Shreveport, La., through Dr. F. Weiss, both of U. S. Bureau of Plant Industry.

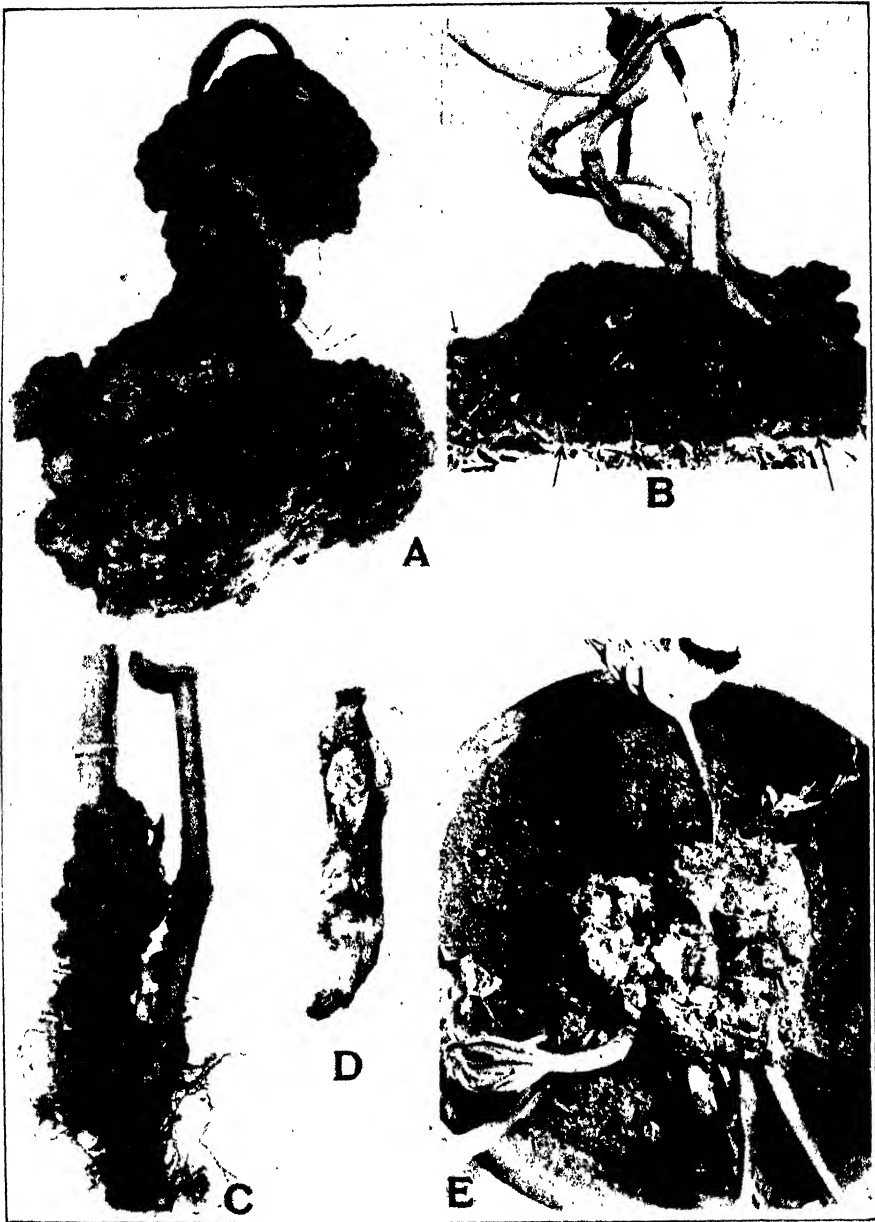


FIG. 1. Giant galls on stems of plants, caused by the root-knot nematode (*Heterodera marioni*). A. Two pieces of such a gall on *Thunbergia grandiflora*. B. The extent of the gall on this *Thunbergia laurifolia* is indicated by the arrows. C. and D. On *Begonia* sp., the latter showing an early stage in the gall development. E. Such a gall on a rhubarb stem (*Rheum raphaniticum*) seen from above.

growth and extreme tissue proliferations, is not yet clear. It may be elucidated by detailed histological and comparative morphological studies of the attacked plants. Figure 1, D, shows a beginning stage in the development of such a gall on the basal stem portion of a begonia. Even this early stage is very unlike the normal root-knot nematode gall on a root; it more closely resembles a crown-gall structure.

The growth of these galls is due mainly to autoinfection, the progeny of the first and succeeding generations of the parasite remaining within the plant. The final result is an enormous accumulation of specimens of the parasite, which, after the final breakdown of the host plant, will invade the surrounding soil.

Another point of interest is the fact that, at least in the case of Thunbergias, the galls usually are fully exposed to the sun. Since the lethal temperature point of larval root-knot nematodes is around 116–117° F., it would appear that autoregulation by the host plant must keep down the temperature of at least the fully insulated portions of the galls, so that this lethal point is not reached. The rugose structure of the galls through increase of the evaporating surface may be favorable to such a function.—G. STEINER, EDNA M. BUHRER, U. S. Department of Agriculture, Washington, and ARTHUR S. RHOADS, Florida Agricultural Experiment Station.

*Paraphelenchus maupasi* Attacks *Hyacinth* Bulbs.—Figure 1 represents a cross section through a hyacinth bulb that exhibited disease symptoms produced by a heavy infestation of *Paraphelenchus maupasi* Micoletzky, 1922. Only a few records of the occurrence of this nematode exist. It was first described by Maupas<sup>1</sup> from Algiers but incorrectly referred to *Aphelenchus agricola*. This classification was corrected by Micoletzky,<sup>2</sup> who first placed it in the subgenus *Paraphelenchus* (1922), which he later<sup>3</sup> (1925) raised to generic rank. De Man<sup>4</sup> described the male but wrongly considered it as that of *Aphelenchus agricola*. In 1929 the writer observed some specimens on coffee roots from Nicaragua. The hyacinth bulbs here described (imported from Holland) apparently represent the first recorded case where this nema was observed in large numbers and was causing definite disease symptoms. As may be seen from the figure, these symptoms

<sup>1</sup> Maupas, E. Modes et formes de reproduction des Nématodes. Arch. Zool. Exper. Sér. 3, 8: 463–624. 1900.

<sup>2</sup> Micoletzky, H. Die freilebenden Erd-Nematoden. Arch. f. Naturgesch. Jahrg. 87: 603. 1922.

<sup>3</sup> ———. Die freilebenden Süßwasser und Moornematoden Dänemarks. Mem. Acad. Roy. Sci. Lettres Danemark, Section Sci., 8me ser. 10: 248–249. 1925.

<sup>4</sup> Man, J. G. de. *Aphelenchus agricola* de Man. In Nouvelles Recherches sur les Nématodes libres terricoles de la Hollande. Capita Zool. Diel. 1: 39–40. Pl. 10, fig. 27–27b. 1921.

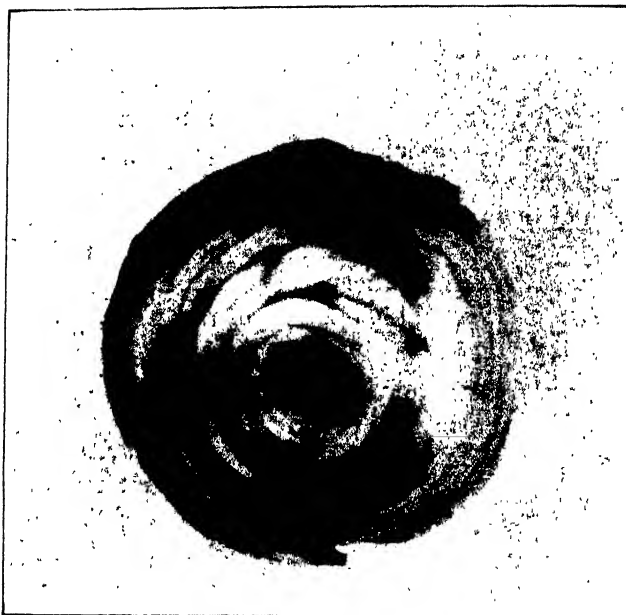


FIG. 1. Cross section of hyacinth bulb infested with *Paraphelenchus maupasi*.

are slightly reminiscent of the ring symptoms as produced by other nemas in bulbs. However, in the present case proper rings are not formed because the nema enters the scales of the bulb and does not usually remain in the space between. The scale tissues are much more broken up than in the case of an infestation by *Anguillulina dipsaci*. The disease appears on a cross-cut scale first as a circular yellowish waxy speck. It seems to progress more by way of segments than by following the rings formed by the scales.

In regard to the nematodes it is of interest that no males were seen, although several hundred females were counted.—G. STEINER and EDNA M. BUHRER, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

*Disease Symptoms Produced by Anguillulina pratensis in Yams.*—Yams from various tropical regions have repeatedly been seen attacked by the meadow nematode (*Anguillulina pratensis* de Man, 1880). In our records the following cases are listed: Three different lots from Japan, one lot each from Jamaica, Porto Rico, and West Africa. The disease symptoms produced by this nematode in yams are small elevations over the entire surface (Fig. 1, A). This figure represents a rather uniformly infested specimen. Under each elevation the tissue containing the nematodes is of brownish discoloration. As the disease advances, the discolored tissue

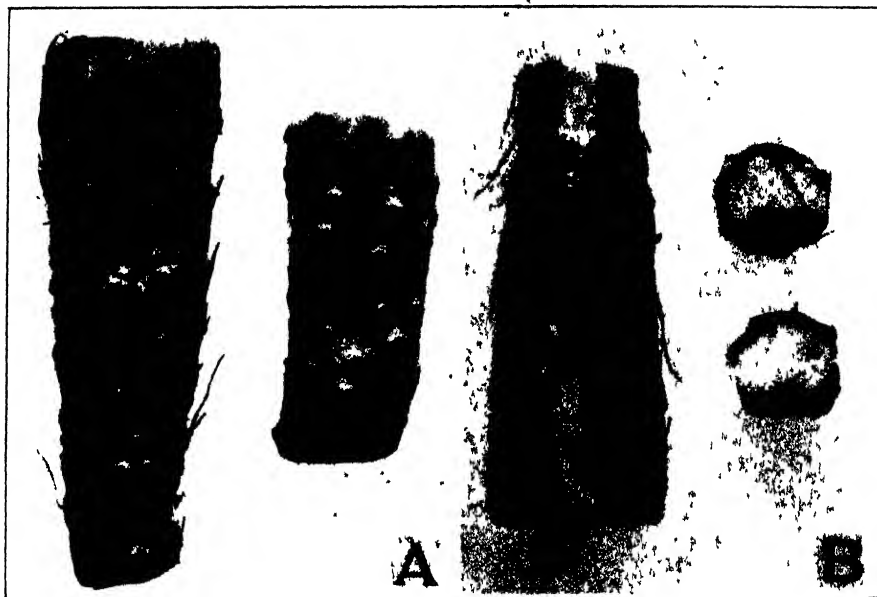


FIG. 1. Yams infested with *Anguillulma pratensis*. A. Surface views. B. Surface view with part of cortex removed to show discoloration beneath surface, and cross sections.

spreads and gradually forms a continuous layer under the surface, penetrating inward simultaneously (Fig. 1, B). General decay then sets in.—(G. STEINER and EDNA M. BUHRER, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.)

*Development of Oats Resistant to Smuts and Rusts.* The development of strains of oats highly resistant to smut, crown rust, and stem rust, and with desirable grain characters, has been a major objective of the Department specialists interested in oat improvement. Sufficient evidence is now at hand to indicate that this objective has been achieved, at least in so far as combined resistance to all three diseases is concerned. During the crop season of 1933 certain hybrid strains were grown in the cooperative breeding nursery at Ames, Iowa, that continued to show resistance to all three diseases. These lines were obtained from a cross of Victoria × Richland.

Victoria was introduced from South America a few years ago, and is highly resistant to or nearly immune from the various physiologic forms of crown rust. It also is extremely resistant to the smuts of oats but is poorly adapted and, so far, has not been sufficiently productive to recommend it for commercial production. On the other hand, the productiveness and

excellent characters of Richland are well-known. However, it lacks resistance to crown rust and to most physiologic forms of smut. Richland is one of the leading commercial varieties of early oats in the Corn Belt. The cross was made by the senior author in the greenhouse at the Arlington Experiment Farm, Rosslyn, Va., in the spring of 1930. The  $F_1$  plants were grown in the cooperative nursery at the Aberdeen Substation, Aberdeen, Idaho, in the summer of 1930 and were not subjected to any artificial inoculations of smut or rust. In the spring of 1931 seed from these  $F_1$  plants was sown in the nursery at Ames, Iowa, where epidemics of crown rust (*Puccinia coronata avenae* (Corda) Eriks. and Henn.) and stem rust (*P. graminis avenae* (Pers.) Eriks. and Henn.) were developed artificially in separate plantings. At harvest those  $F_2$  plants that showed resistance to one or both rusts were isolated and stored for further study. Seed from these  $F_2$  plants was sown in progeny rows at Ames, Iowa, in the spring of 1932. The  $F_3$  plants were subjected to an artificially produced epidemic of the two rusts, as in the  $F_2$  generation. Before sowing, the seed was inoculated with spores of loose smut of oats (*Ustilago avenae* (Pers.) Jens.) for a test of resistance to this disease also. At harvest several of the  $F_3$  lines showed freedom from smut and appeared to be homozygous for resistance both to crown rust and stem rust.

Seed from several of these more promising  $F_3$  lines was sent to Washington, D. C., for sowing in the greenhouse at Arlington Farm in the fall of 1932. The hulls were removed and the seed was blackened with spores of loose smut of oats collected in the vicinity of Ames, Iowa, in the summer of 1932. Immediately after sowing the seed the temperature of the greenhouse was raised for about a week to facilitate inoculation. In February, 1933, artificially induced epidemics of both crown and stem rust were developed on separate seedlings in the greenhouse at Arlington Farm. The inoculation was accomplished by placing the plants in a large glass incubation chamber where they were sprayed with water and dusted with rust spores. They were then allowed to remain in the chamber for 24 hours.

An excellent infection of smut and of the 2 rusts was obtained. Of the several families grown, a few showed a combination of resistance to all 3 diseases. In 1933, selections from these families were tested for resistance to all 3 diseases at Ames, Iowa, where, under field conditions, they continued to show marked resistance to the rusts and smuts.

Heavy epidemics of crown and stem rust were developed in the field each year by the aid of artificial inoculations. Urediospore suspensions of physiologic forms of crown and stem rust, common throughout the North Central States, were hypodermically injected into susceptible plants growing in check rows. These inoculations were initiated when the plants were 8 to 10 inches high and resulted in their complete infection in the check and



border rows when they reached the boot-stage of development. The rust disseminated from these infected rows, combined with the natural infection present, was sufficient each year to produce a maximum infection on all susceptible plants in the nursery.

A second group of selections showing a combination of resistance to all 3 diseases has been developed from the cross Victoria  $\times$  Richland. Remnant seed from the  $F_1$  plants grown at Aberdeen, Idaho, in 1930 was sown in the greenhouse at the Arlington Farm in the fall of 1931. The  $F_2$  plants were inoculated with urediospores of crown and stem rusts by the method previously described, but, owing to unfavorable conditions, low percentages of infection were obtained. However, progenies from those lines that showed some resistance to the rusts were grown at Aberdeen in 1932 from seed blackened with inoculum containing several physiologic forms of both smuts (*Ustilago avenae* and *U. levis*). At harvest all progenies showing smut infection were discarded. Seed from the remaining plants was hulled and inoculated with smut spores and sown in the greenhouse at Arlington Farm in the fall of 1932. The  $F_3$  plants were subjected to artificial epidemics of both rusts. Excellent infections of both smuts and both rusts were obtained and many plants succumbed; a few families, however, showed high resistance to both rusts and were smut-free. These surviving families were further tested for resistance to all 3 diseases at Ames, Iowa, in 1933 under epidemic conditions in the field. Their descendants continued to show a combination of resistance to crown and stem rust and smut, thus confirming the results obtained from the material continuously tested under field conditions at Ames.

Since the tests of the selections from the cross Victoria  $\times$  Richland were much more severe in the greenhouse and field than under average field conditions, it is believed that the desired end result, that is, the development of strains possessing a combination of resistance to all 3 diseases has been accomplished. However, it is still too soon to say whether these new oats will be suitable for adoption by the farmer. Their adaptation and productiveness remain to be determined.—T. R. STANTON, H. C. MURPHY, F. A. COFFMAN, and H. B. HUMPHREY, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

*The Relative Importance of Cercospora herpotrichoides and of Leptosphaeria herpotrichoides as Parasites of Winter Cereals.*—Until the last few years *Leptosphaeria herpotrichoides* de Not. was generally accepted as the primary parasite involved in the "straw-breaker" foot rot of winter wheat and winter rye in Europe. Recently, in the Pacific Northwest, C.

*herpotrichoides* Fron was shown to be the cause of a straw-breaker type of foot rot of winter wheat.<sup>1</sup>

Foëx and Rosella<sup>2</sup> determined that a fungus, later found to be *Cercospora herpotrichoides*, was an active parasite of wheat in France.

Recently, Schaffnit,<sup>3</sup> following the work in the United States and in France, found that *C. herpotrichoides* was the cause of the straw-breaker foot rot of cereals in Germany.

In the Mycological Collections of the Bureau of Plant Industry, U. S. Department of Agriculture, at Washington, D. C., *Cercospora herpotrichoides* occurs on specimens of diseased wheat collected in Norway by E. Bachala and E. C. Stakman and labeled *Leptosphaeria herpotrichoides*. From conversation with Dr. Olaf Aamodt of the University of Alberta, who has seen the straw-breaker foot rot in Sweden, it is evident that the symptoms of the straw-breaker disease in Sweden are practically identical with those in the United States in Washington, Oregon, and Idaho.

While *Cercospora herpotrichoides* is an active parasite, the writer has not found *Leptosphaeria herpotrichoides* to be so. Pure cultures of *L. herpotrichoides*, kindly furnished by Foëx from France and by Henry from Canada, have been used in field and greenhouse inoculation studies in the same manner as was *Cercospora herpotrichoides*.<sup>4</sup> *L. herpotrichoides* grew saprophytically over the basal parts of winter wheat culms and penetrated into the outer leaf sheaths to some extent, but even under the favorable conditions of the experiments it was only a weak parasite.

The writer wishes to point out the need for re-study of the straw-breaker disease in those European countries where no recent work has been done with this malady. It has been the writer's belief for some years that *C. herpotrichoides* is the cause of most of the straw-breaker disease of wheat attributed to *L. herpotrichoides* in Europe.—RODERICK SPRAGUE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Oregon and Washington Agricultural Experiment Stations.

<sup>1</sup> Sprague, R. *Cercospora herpotrichoides* Fron, the cause of the Columbia Basin foot rot of winter wheat. *Science*, n. s. 74: 51-53. 1931.

<sup>2</sup> Foëx, Et., and Et. Rosella. Sur les diverses formes du piétin. *Rev. Path. Veg. et Ent. Agr.* 17: 41-51. 1930.

<sup>3</sup> Schaffnit, E. Beiträge zur Kenntnis der Fusskrankheiten des Getreides (I. Mitteilung). *Cercospora herpotrichoides* (Fron) als Ursache der Halmbruchkrankheit des Getreides. *Phytopath. Ztschr.* 5: 493-503. 1933.

<sup>4</sup> Sprague, R., and H. Fellows. The *Cercospora* foot rot of winter cereals. U. S. Dept. Agr. Tech. Bull. (In press.)

## BOOK REVIEW

*Traité de Pathologie Végétale.* By G. Arnaud and Madeleine Arnaud. Tome I. Vols. I, II, and Atlas. (*Encyclopédie Mycologique*, Tomes III, IV, V), 1831 pp., 702 figs., 34 col. plates. Paul Lechevalier et Fils, Paris. 1931. 750 fr.

This splendid work on plant pathology of about 1900 pages (including the Atlas) forms Tome I (Volumes I and II, and Atlas) of the authors' treatise, and is also Tomes III, IV and V, of Lechevalier's *Encyclopédie Mycologique*. It is profusely illustrated with 702 original figures and with 34 colored plates by Mme. S. Ballings. These plates are bound together to form the Atlas. The treatise describes over 400 diseases of the grape vine, fruit trees and small fruits, including subtropical fruits grown in the Mediterranean region. Besides the 400 diseases described more or less fully, many parasitic fungi and bacteria of little or no economic importance, especially in France, are briefly noted. The insects that attack the crops mentioned above and methods for their control are also briefly described. The space allotted to each disease varies from a paragraph to 79 pages (downy mildew of grapes), according to the importance of the crop and of the disease in France. However, all the known parasites attacking the crops mentioned above, in whatever part of the world they occur, are mentioned or described.

The treatise has an introduction of 106 pages. It deals with the history of plant pathology, the classification of diseases according to symptoms, the nature and classification of the parasites (Phanerogamic plants, fungi, bacteria and insects), general control methods, and climatology. It aims to give readers, who are not plant pathologists, sufficient technical knowledge to enable them to understand the cause of disease and the principles underlying control measures, and thus fully profit from the treatment of the diseases in the pages that follow. The introduction is concluded with a long list of monographs on general plant pathology mostly in French, English, German and Italian. Throughout these volumes, each section is followed by an extensive and up-to-date bibliography.

The remainder of Volume I is devoted to the diseases of grapes (543 pages), to a chapter devoted to general considerations on diseases of fruit trees (belonging to the Rosaceae), and to the diseases of apples (335 pages). Volume II deals with the diseases of pear, quince, medlar-tree, mountain ash, hawthorn, peach and almond, apricot, plum, cherry, currant and gooseberry, raspberry and blackberry, cranberry, strawberry, olive, citrus trees, fig, mulberry, pomegranate, loquat, Japanese persimmon, carob, pistachio-tree, jujube-tree, and date-palm. The sub-tropical plants are grouped together under the heading of Mediterranean trees and crops.

The subject-matter is arranged according to the host under its French common name. Common names in English, German and Italian, and the scientific name are also given. In the case of the most important crops, the history of their culture, their economic importance and their botanical classification are dealt with fully. For instance, 95 pages are devoted to these subjects pertaining to the grape vine, and 18 pages to those of the apple. Then follows a list of diseases and insects affecting the host, and a discussion on general control methods. Then, after this, each disease is taken up separately, those of greatest economic importance being dealt with first, and at considerable length. The rest of the diseases attacking that host are grouped as follows: the fungous diseases attacking the leaves, those attacking the fruit, the branches and trunk, and the roots; the bacterial diseases; parasitic insects; non-parasitic (physiological) diseases; and virus diseases.

Each disease is dealt with in a separate section of varying length under the heading of its French common name. Common names in English, German and Italian, and the scientific name and authority are also given. If it is an important disease, its history and geographical distribution, and the susceptibility of varieties to it, are discussed. Then follows the symptomatology, conditions favorable for its development, control measures, and a systematic and morphological study of the organism. The nomenclature receives due consideration, all the synonyms being given, together with their authority. In most cases, a reference to the original description of the organism is included in the bibliography at the end of the section.

These volumes are written in simple, but beautiful French, in order that they may be read by growers and specialists alike. They are therefore easily readable to those with only a slight reading knowledge of this language. They are remarkably free from typographical errors, and are rich in original illustrations and in the extensive and up-to-date bibliographies at the end of each section, throughout the books. The exhaustive treatment of the subjects, the original illustrations, and the extensive bibliographies make this work of great value both as a text-book to the student and as a work of reference to the advanced student and investigator, and should be in the library of every botanical and agricultural institution. The authors are to be congratulated in putting out a work of such size and quality, and on their large number of entirely original illustrations. It is hoped that similar volumes on cereal and vegetable diseases will soon follow these.—H. N. RACICOT, Division of Botany, Central Experimental Farm, Ottawa, Canada.

Announcement

VI INTERNATIONAL BOTANICAL CONGRESS

(Secretary: DR. M. J. SIRKS, Wageningen, Holland)

Wageningen November 6th. 1933

Dear Sir,

The Executive Committee of the VI International Botanical Congress will be much obliged for your kind publishing free of charge the enclosed second press-notice in your journal.

With sincerest thanks, yours faithfully,  
the secretary of the Executive Committee  
(DR. M. J. SIRKS).

VI INTERNATIONALER BOTANISCHER KONGRESS

(Schriftführer: DR. M. J. SIRKS, Wageningen, Holland)

Wageningen am 6 November 1933.

Sehr geehrter Herr,

Der Vorbereitungsausschuss des VI Internationalen Botanischen Kongress wird Ihnen für eine kostenlose Veröffentlichung der einliegenden zweiten Presse-Mitteilung in Ihrer Zeitschrift sehr verbunden sein.

Mit bestem Dank im Voraus, Hochachtungsvoll,  
der Schriftführer des Vorbereitungsausschusses  
(DR. M. J. SIRKS).

VI CONGRÈS INTERNATIONAL DE BOTANIQUE

(Secrétaire: DR. M. J. SIRKS, Wageningen, Hollande)

Wageningen, ce 6 novembre 1933.

Monsieur,

Le Comité d'Organisation du VI Congrès International de Botanique vous sera bien obligé de la publication gratuite dans votre périodique de la notice ci-jointe, la seconde notice pour la presse.

Veuillez accepter, Monsieur, nos remerciements  
anticipés,  
le secrétaire du Comité d'Organisation,  
(DR. M. J. SIRKS).

## VI INTERNATIONAL BOTANICAL CONGRESS

Amsterdam, September 2d.-7th. 1935

The Organizing Committee of the VI International Botanical Congress from various sides has been asked to change the dates of this Congress; the Committee has now decided that the Congress will meet at Amsterdam (Holland)

SEPTEMBER 2D.-7TH. 1935

A first notice regarding this Congress has been sent out to a number of addresses; for additional copies please apply to the secretary, DR. M. J. SIRKS, Wageningen, Holland.

## VI INTERNATIONALER BOTANISCHER KONGRESS

Amsterdam, 2-7 September 1935

Der Vorbereitungsausschuss des Sechsten Internationalen Botanischen Kongresses ist von verschiedenen Seiten gebeten worden die Daten des Kongresses zu ändern; demzufolge ist jetzt beschlossen worden, den Kongress in Amsterdam abzuhalten

vom 2 bis zum 7 September 1935.

Eine erste Notiz über den Kongress ist an manche Adressen versandt worden; weitere Exemplare können vom Sekretär, DR. M. J. SIRKS in Wageningen (Holland) bezogen werden.

## VI CONGRÈS INTERNATIONAL DE BOTANIQUE

Amsterdam, 2-7 septembre 1935

En conséquence de prières lui venues de divers côtés le Comité d'Organisation du Sixième Congrès International de Botanique a résolu de changer les dates du Congrès à Amsterdam et de les fixer à

2-7 septembre 1935.

Les premières communications concernant le Congrès ont été expédiées. Prière de s'adresser au Secrétaire, M. le Dr. M. J. SIRKS, Wageningen, Hollande, pour en obtenir d'autres exemplaires.

# PHYTOPATHOLOGY

VOLUME 24

NUMBER 3

MARCH, 1934

## CONTROL OF PHYTOPHTHORA HEART ROT OF PINEAPPLE PLANTS<sup>1</sup>

F. P. MEHRlich

(Accepted for publication April 26, 1933)

### INTRODUCTION

This paper presents the results of 2 years of studies designed to give an effective and economical control of heart rot of the pineapple plant, which in Hawaii is caused by *Phytophthora cinnamomi* Rands, *Ph. palmivora* Butl. (*Ph. meadii* McRae), and *Ph. parasitica* Dastur. The last-named species is interpreted to include *Ph. melongenae* Sawada. Heart rot caused by each of these 3 species is so closely similar that, for present purposes, no distinctions are necessary. Details of the local and wide-spread geographic distribution of the pathogens, their physiology, morphology, and taxonomy, in addition to information concerning the epidemiology of the disease, are presented in a separate paper (12). A prior report of heart rot of the pineapple plant in Hawaii, not including control measures, has been presented by Sideris and Paxton (13).

### The Disease and Its Occurrence

Heart rot, as suggested by the name, is a rot of the heart of the plant, which destroys the younger portions of the stem and the bases of the central leaves. The outermost leaves characteristically escape invasion, as do the older parts of the stem. In very young plants, however, all of these regions may be involved. Heart rot also is known as top rot (15).

In the field heart rot of young plants is recognized by a series of color changes of the leaves, a lack of growth of the plant, and a ready removal of the entire heart of the plant when the central leaves are lightly pulled. The leaves of a diseased plant first become reddish, but soon change to a pallid yellow. Before the color change is apparent, the bases of affected leaves display a transparent water-soaked area. After the change in color is noticeable the leaf base will be found to display a malodorous, yellow-white rotted area, bordered by a very distinct and characteristic brown margin (Fig. 1, B). This area ordinarily extends into the stem tissue below, but is limited quite sharply by the green parts of the leaf above. The invaded stem tissue is of a cheesy consistency. It charac-

<sup>1</sup>Published with the approval of the Director as Technical Paper No. 50 of the Experiment Station of the Association of Hawaiian Pineapple Cannerys, University of Hawaii.

teristically is similar to the leaf lesions in odor, color, and possession of the well-defined brown margin. Plants in advanced stages of heart rot may display a falling apart of the leaves. The older basal parts of the stems of larger plants usually are not rotted by *Phytophthora* spp. and may remain sound. Secondary organisms, both fungi and bacteria, rapidly follow the casual organism, decomposing the invaded tissues changing the odor from that characteristic of the disease.

The phase of heart rot described above agrees with that mentioned in the scant literature regarding the disease (1, 2, 5, 7, 8, 11, 13, 15). It is this phase with which the present study is concerned. Another phase of

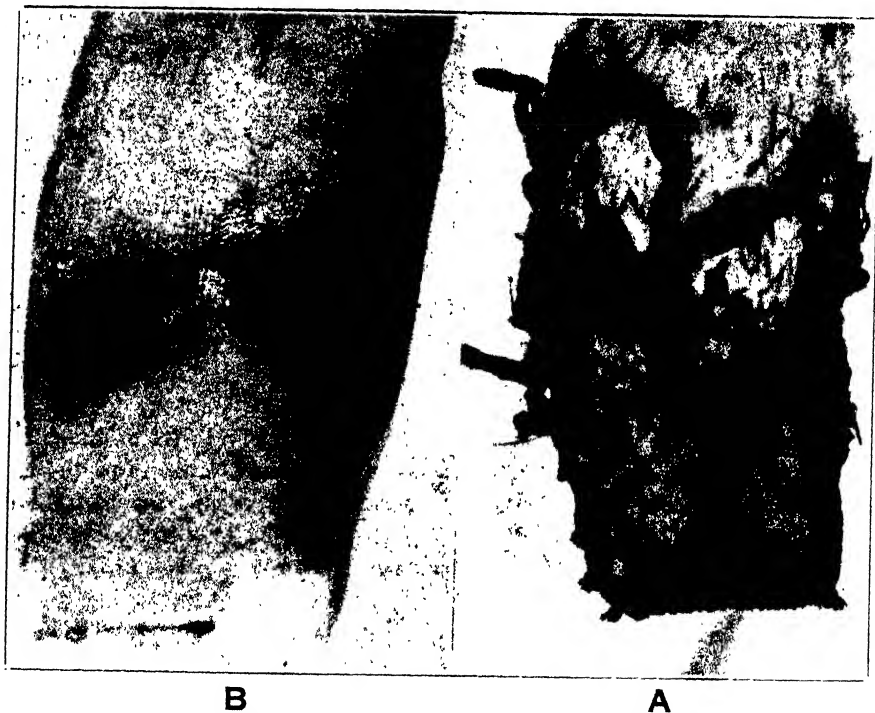


FIG. 1. Pineapple heart rot caused by *Phytophthora cinnamomi*. Note the rotted white areas bordered by the characteristic brown margin. A. Stem lesion. B. Leaf lesion.

the disease, not reported by others, affects older, more mature plants. Plants old enough to flower and even those which have already borne fruit may succumb (Fig. 2). The lesions displayed by the stem and leaves of older plants are similar in all respects to those described above for younger plants. The central leaves of such diseased plants, being separated from their vascular supply, lose their turgidity, collapse, and give the plant a prostrate appearance. The older, more fibrous parts of the stem are rarely



invaded. They may give rise to new growth from previously dormant axillary buds either above or below the soil. This new growth may remain healthy, reestablishing the plant, or may succumb. Suckers from healthy plants may be killed by heart rot without any apparent effect on the parent plant. Stem lesions in older plants do not always involve the leaves. In such cases the plant becomes stunted and the narrower, stiffer leaves show an abnormal reddening.

The losses from heart rot of young plants occur intensively chiefly within very limited regions of large fields. In the affected areas, ordinarily

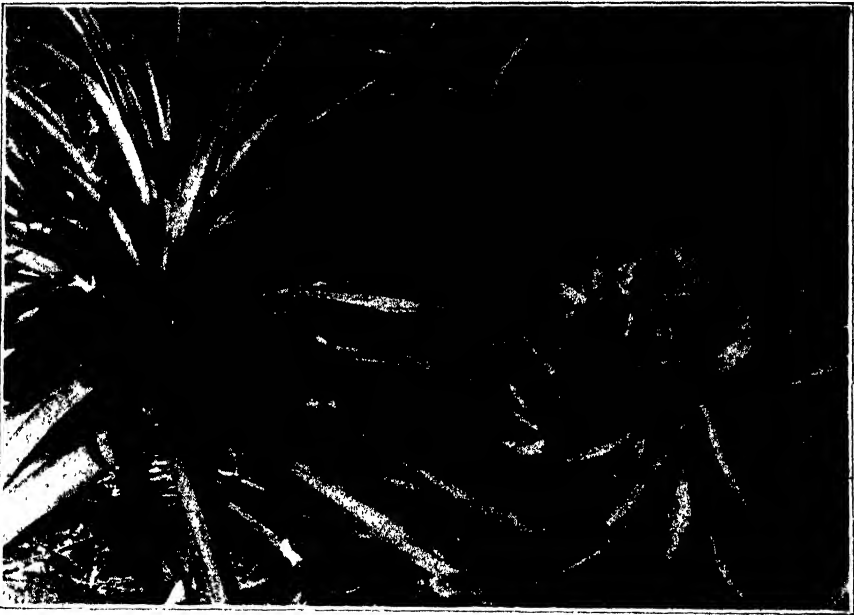


FIG. 2. Near view of a pineapple plant failing from heart rot after having borne fruit, compared with an adjacent healthy plant of the same age. The shoot in the foreground as well as the slips all show the results of infection.

two acres or less in extent, 50 to 75 per cent of the plants may be destroyed. Losses of more than 15 per cent of all the plants in a 100-acre field have been observed, but such wide-spread destruction is characteristic only under the most adverse conditions. Poor physical condition of the soil, inadequate drainage, and flooding intensify the losses. Figure 3 shows a characteristic heart-rot area, where large vacant spaces have resulted from the removal of diseased plants.

Within the territory of Hawaii the disease has been observed on the islands of Oahu, Molokai, Lanai, Kauai and Maui. It has been found in soils ranging in pH from 3.5 to above 7.0. It occurs in areas with an an-

mual rainfall in excess of 100 inches and, as well, in areas averaging less than 20 inches a year. In regions where the disease reaches its maximum severity, light showers characterize the usual day, and days with no precipitation are infrequent. At the sites of experiments 7 and 8, reported below, 30 inches of rain fell within a month and 15.5 inches were recorded for a single day.

#### Former Attempts at Control

Sideris and Paxton (13) recommended improvement of soil drainage and aeration for limiting losses from *Phytophthora* spp. in Hawaii but presented no experimental evidence of the efficiency of such measures. In many areas such improvement alone is ineffective as a control.

Johnson (6) states that Ashby found spraying with hot Bordeaux beneficial in Jamaica and that Siggers in Costa Rica used a lime sulphur dust.



FIG. 3. Molokai control experiment after 5 months. The plots are, left to right: Check, no treatment; Bordeaux dip 1-0.7-3; check; Niagara colloidal sulphur dust.

Methods of application, concentrations used, or detailed results of control effected are not presented.

In 1930 M. B. Linford and G. E. Paxton, of this Station, undertook preliminary studies of control by fungicides. Their unpublished notes suggest that, to be most effective, fungicides should be applied to the planting material before setting it in the field, or that the newly set plants should be immediately treated with quantities of liquid fungicides sufficient to wet the soil around the butt of each plant. They used representative copper and mercury fungicides, aluminum sulphate, sulphuric acid, and a colloidal sulphur in limited field tests and concluded that Bordeaux mixture, Burgundy mixture, Semesan, aluminum sulphate, and copper carbonate all showed some promise.

### Requirements of a Successful Control Practice

A control measure, to be successful, must take into account a number of separate factors: it must protect the susceptible tissues both above and below ground and be a single application, if possible. It must be of a lasting nature to withstand heavy rains and possible flooding and must be adaptable to unpredictable major or minor occurrences of disease of local or widespread distribution.

The causal organisms all survive for long periods of time in the soil, whence they enter the host through wounds mostly below the surface of the soil. In rare cases wounds near the apex of the stem may serve as avenues of entrance. Evidence of penetration through sound tissues is yet inconclusive, although experimental studies suggest that *Phytophthora cinnamomi* may enter directly under certain conditions.

Standard planting practices in the Hawaiian Islands involve the use of 10,000 to 20,000 pineapple plants per acre. Only a small percentage of these, distributed over a large area, may become diseased, but the total numbers of plants treated in a single field may be many thousands. Because of the distances involved repeated treatments of any nature would involve high costs for labor.

The magnitude of losses from heart rot is dependent upon environmental factors, which may vary considerably within a given field from year to year; upon the age, vigor, and type of planting materials; and upon distribution, abundance, and species of the parasite. The varied types of information requisite to a prediction of losses in any locality are not now available.

### EXPERIMENTAL STUDIES

Studies started by the writer in the fall of 1930 were designed to determine the concentrations of representative fungicides that could be placed on the tender tissues of planting materials and young growing plants without causing undue injury and to test under field conditions the controlling effect of various of the most desirable preparations.

Experiments to determine fungicides desirable for control tests in the field were carried on first in the laboratory and later in outdoor plots. Only the field tests will be reported.

To test plant tolerance to various fungicides, young, vigorously growing plants were treated in blocks of 6 to 10 each. Two grams of dust were sifted into the hearts and onto the leaves, or 250 cc. of freshly prepared liquid was poured into the hearts of each plant treated.

Table 1 presents for each fungicide only the highest concentration tested and tolerated, or the various significant ratios of ingredients that did not injure the test plants. Fungicides causing serious injury at the lowest concentrations used are not included in the table. The Bordeaux

TABLE 1.—*Fungicides tested and tolerated by young growing pineapple plants*

Fungicide	Concentration	Fungicide	Concentration
<i>Dusts</i>			
Niagara colloidal sulphur	2 gm. per plant	Bayer Special II-6	2 gm. per plant
Lime sulphur	2 gm. per plant	Fungi Bordeaux	2 gm. per plant
Sublimed sulphur	2 gm. per plant	Copper carbonate	2 gm. per plant
Ferrox sulphur	2 gm. per plant		
<i>Liquid in Hearts</i>			
Permek sulphur	1-2.5	Bordeaux	1-0.35-1
Ialine	1-5	"	1-0.7-1
Nickel	1-1.2	"	1-1.05-1
Lime	1-11	"	1-0.7-1.5
" " + alumi-			
num sulphate	1-0.65-15	"	1-0.7-2
Calogren + Kayso (20 g./gal.)	1-4.5	"	1-1.05-2
Copper carbonate	1-7	"	1-0.7-2.5
" " + sal soda	1-0.75-11	"	1-1.05-2.5
" " + ammo-			
nium carbonate	1-2-11	" + resin sticker	1-0.35-14
Copper carbonate + ammo-			
nium carbonate	1-3-15	" + " "	1-0.7-14
Copper carbonate + hy-			
drated lime	1-2-11	" + iron sticker	1-0.7-3
Copper carbonate + hy-			
drated lime + Kayso 20		" + Goulac	
g./gal.	1-1-15	sticker	1-0.7-2.5
Pyrox	1-2.5	" + Kayso	
		sticker	1-0.7-2.5

mixtures used were made with fresh hydrated Waianae lime, a local lime produced at Waianae, Oahu, T. H.<sup>2</sup>

<sup>2</sup> An analysis of the lime used in the Bordeaux mixtures of tests described below is as follows:

<i>Analysis</i>	<i>Per cent</i>
Silica (SiO <sub>2</sub> )	1.03
Iron and alumina (Fe <sub>2</sub> O <sub>3</sub> and Al <sub>2</sub> O <sub>3</sub> )	1.46
Calcium oxide (CaO)	72.67
Magnesia (MgO)	1.53
Sulfates (SO <sub>3</sub> )	0.15
Carbon dioxide (CO <sub>2</sub> )	1.10
Moisture (H <sub>2</sub> O)	0.69
Loss on ignition (Less CO <sub>2</sub> )	22.06
Available CaO (Sugar soluble)	68.2
Fineness:	
Residue on a No. 30 sieve	.0098
Residue on a No. 200 sieve	.78

In all references to the Bordeaux mixtures used, the lime content will be expressed as the available calcium oxide equivalent of the hydrated lime; *i.e.*, a factor of 0.7 will be used.

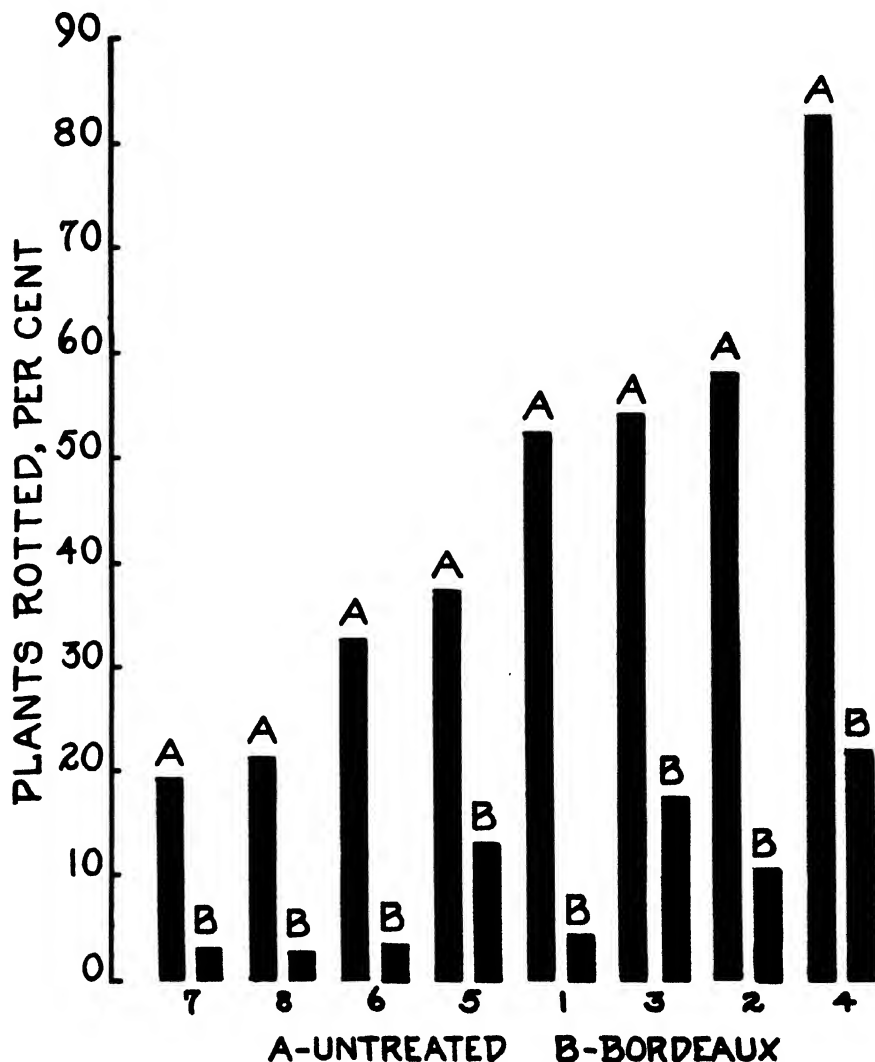


FIG. 4. Control of pineapple heart rot by Bordeaux 1-0.7-3 used as a dip. Numbers below the pairs of columns refer to the experiments discussed in the text. Experiments were conducted on 3 different islands.

#### Field Control Studies

During the planting seasons of 1930-1931, and 1931-1932, a number of field tests of half-acre or acre size were installed on the islands of Oahu,

Lanai, Molokai and Kauai. In a number of these experiments disease did not develop significantly in the check plots. In other instances complicating factors completely invalidated the data. Only those tests are reported where the effect of fungicides on the incidence of disease could be evaluated accurately.

*Experiment 1:* Factors of coverage, adherence, ease of preparation, lack of toxic effects to the host, and cost were considered in the selection of representative fungicides for the first field control test.

The site selected was characterized by repeated losses from heart rot, and by stunting of plants from root failure, both caused by *Phytophthora cinnamomi*. Drainage was poor, the surface soil shallow, and the subsoil compact and gummy. Planting material was large slips, except that 10 plots of crowns were also used as indicated below.

All slips except the checks were either dipped or dusted prior to planting. Dusts were applied by rotating the planting material in a barrel into which the fungicidal dusts were blown. The liquids were freshly mixed in the field; in them the appropriate plants were immersed momentarily.

There were 175 plots of 2 rows (one bed) each, averaging 33 feet long and containing about 70 plants. They included 9 replications of each of 15 treatments, 30 plots of untreated slips, and 10 plots of untreated crowns as controls. The 9 replications were in 3 series of 3 replications each, and these series were located in separate parts of the experimental area.

In this experiment, as in all others detailed below, counts of plants infected with heart rot were made periodically by lightly pulling the center leaves of each plant. All diseased plants were removed from the field when discovered. Six months from the time of planting, the active development of heart rot had ceased. Later counts revealed an occasional plant which was infected, but the numbers were insignificant. Heart rot developed to a significant degree in parts of the field occupied by series 1 and 2, but not series 3. This latter series was omitted, therefore, from computation of results.

Table 2 shows the composition of each fungicide used and, for series 1 and 2 only, the numbers of plants treated, the percentage of rot in the treated plots, the percentage of rot in adjacent checks, and that of the control effected by the treatments. Control was calculated from the percentage differences of disease in the treated plots and adjacent checks.

This test indicated that fungicides of many types may give a high degree of control if used in proper strength. Certain of the other preparations showed a high degree of control, but were toxic to the plants. Thus severe burn was apparent in plots treated with copper carbonate dust, dry lime sulphur dust, and copper carbonate aqueous suspension, but the injury was

not permanent in the lime sulphur plots. Burning by copper carbonate dust and copper carbonate liquid involved 81 and 55 per cent of the plants, respectively. The addition of hydrated lime to each reduced the burning of the dust to 37 per cent and of the liquid to 38 per cent. See treatments 8 and 12 in table 2.

TABLE 2.—*Results from heart-rot control experiment 1, showing the compositions of fungicides used, the total plants in each treatment, the percentage of rot in treated and check plots, and the percentage of control effected*

No.	Fungicide	Plants treated	Rot in treated plots	Rot in checks	Control
	Composition				
<i>Dips</i>		<i>No.</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1	Bordeaux 1-0.7-6	704	4.74	52.39	90.95
2	" 1-0.7-13.5 plus resin sticker	688	8.08	42.14	80.83
3	" 1-0.7-13.5 plus no sticker	678	6.71	39.86	83.17
4	" 1-0.35-13.5	695	11.90	39.76	70.08
5	" 1-0.35-13.5 plus resin sticker	675	8.01	37.80	78.81
6	Ialine sulphur 1-3	654	54.29	49.67	- 9.3
7	Copper carbonate 1-12	709	2.65	48.59	94.55
8	" " plus hydrated lime 1-0.25-15	678	3.96	51.86	92.36
<i>Dusts</i>					
9	Bayer special II-6	702	15.06	32.36	53.47
10	Lime sulphur (dry)	640	3.75	51.0	92.65
11	Copper carbonate	692	0.0	38.57	100.00
12	" " plus hydrated lime 4-1	693	0.0	45.61	100.00
13	Ferrox sulphur	730	1.10	38.28	97.13
14	Fungi Bordeaux	699	0.0	34.58	100.00
15	Niagara colloidal sulphur, X compound	668	1.34	47.43	97.18

*Experiment 2:* The first control experiment of a significant size, using a concentration of Bordeaux higher than 1-0.7-6, was installed at Kipu, Molokai, January, 1931. In the area selected for this test more than 50 per cent of the plants had died of heart rot caused by *Phytophthora parasitica*. The remaining plants were removed. Plots, numbered 1 to 17 each, consisted of 4 adjacent rows of plants. The planting material used was well-cured crowns. From some of these the basal leaves were stripped before curing. Others were cured, treated, and planted without stripping.

Eight months after installation the percentage control effected by the

various treatments was calculated. Table 3 presents these data, also the number of replications of each treatment and their distribution. An analysis of the control data by Student's method for paired observations (10) showed that differences noted between Ferrox and Niagara sulphurs on the one hand, and between trimmed and untrimmed crowns on the other are not statistically significant. Differences between other treatments are highly significant. Figure 3 illustrates this experiment 5 months after planting.

TABLE 3.—Control of heart rot by fungicides at Kipu, Molokai. The distribution of plots, the percentage of plants of each plot that rotted, and the percentage control of each fungicide is shown

Nontreated check		Bordeaux Dip 1-0.7-3 <sup>a</sup>				Niagara Colloidal Sulphur		Ferrox Sulphur	
Plot No.	Per cent of plants rotted	Trimmed		Untrimmed		Row No.	Rotted plants	Row No.	Rotted plants
		Row No.	Rotted plants	Row No.	Rotted plants				
			Per cent		Per cent		Per cent		Per cent
1	51.22	2	7.14	6	12.28	3	69.12	5	48.48
4	50.59	6	27.27	11	5.71	8	54.26	9	65.41
7	66.67	11	10.00	15	15.49	12	64.34	14	60.40
10	62.77	15	12.16					17	70.99
13	63.70								
16	49.37								
Percentage control by fungicides:									
		75.57		84.42		- 11.78		- 8.19	

<sup>a</sup> Trimmed and untrimmed crowns were planted in the same beds; trimmed ones were placed in the 2 left rows and untrimmed ones in the 2 right rows.

*Experiment 3:* During March, 1932, a half-acre test of 4 liquid fungicides and a dust was installed at Opaëula, Oahu. The liquids were applied by dipping, as in earlier tests, but the dust, Ferrox sulphur, was applied with a hand duster of the blower type. This experiment occupied an area where heart rot and root rot caused by *Phytophthora cinnamomi* had killed about 70 per cent of the first planting. Soil conditions were comparable to those in experiment 1 described above.

A modified Latin Square arrangement of plots was used, incorporating twice as many check plots as those of any single treatment. Each plot was 18 feet long and 2 beds wide. Approximately 600 plants were treated with each fungicide. The planting materials, having stood on the trimming grounds for many months, were of poor quality.



The pertinent data from experiment 3 are presented in tables 4 to 7. Table 4 shows the distribution of the plots of each treatment and gives the

TABLE 4.—*Showing the distribution of plots in experiment 3, and the percentage of disease in each plot after 6 months<sup>a</sup>*

A 30.2	C 50.0	E 67.3	G 21.4	F 14.1	B 15.2	D 26.4
G 46.3	B 16.3	F 19.7	A 50.0	D 27.6	E 52.9	C 59.8
E 56.3	F 28.1	A 43.0	D 29.3	C 47.6	G 25.5	B 21.1
B 25.3	D 52.4	G 52.2	F 18.4	A 91.3	C 57.7	E 64.3
F 44.7	A 78.6	B 14.5	C 57.9	E 68.1	D 24.7	G 37.5
D 30.3	G 18.9	C 56.0	E 58.0	B 18.9	A 44.9	F 18.8
C 64.9	F 54.0	D 5.6	B 14.8	G 25.0	F 20.7	A 29.6

<sup>a</sup> Treatment: A and C, nontreated checks; B, Bordeaux dip 1-0.7-3; D, Ferrox sulphur dust; E, Bayer Special II-6; F, copper carbonate with lime dip 2-1-15; G, Ansul wettable sulphur dip 1-6.

percentage of diseased plants in each plot after 6 months. From these data the control effected by the various treatments was calculated (Table 5).

TABLE 5.—*The composition of fungicides, the numbers of plants treated, the mean percentage of diseased plants in each treatment, and the control effected by the fungicides in experiment 3*

Treatment	Plants treated	Mean per cent rot	Control effected
A & C Nontreated check . . . . .	1109	54.4	
B Bordeaux dip 1-0.7-3 . . . . .	546	18.0	66.92
D Ferrox sulphur dust . . . . .	598	28.0	48.53
E Bayer II-6 dip 1-3 . . . . .	600	60.1	-11.05
F Copper carbonate plus hydrated lime 2-1-15 . . . . .	584	23.5	56.81
G Ansul wettable sulphur 1-6 . . . . .	531	32.4	40.45

Fisher's method for the analysis of Latin Square experiments permits an evaluation of the significance of the experiment as a whole, as well as of the individual treatments. Table 6 is the analysis of variance of experiment 3 by Fisher's method (4).

TABLE 6.—*Analysis of variance of experiment 3*

Source of variance	Degrees of freedom	Sum of squares	Variance	$\frac{1}{2}$ log. variance
Rows .....	6	2506.3	417.7	3.0180
Columns .....	6	513.4	85.6	2.2239
Treatments .....	5 <sup>a</sup>	12347.3	2469.5	3.9066
Remainder .....	31	3700.2	119.4	2.8917
Total .. .....	48	19067.2		

<sup>a</sup> The identical treatments A and C are considered as a single check in this analysis.

The variance due to treatments is very significant for the difference between the values  $\frac{1}{2}$  log. variance of the treatments and remainder greatly exceeds the value .4164, which is the deviation that would be exceeded by chance in 5 per cent of random samples (See (3), table VI).

The significance of variance between the several treatments of experiment 3 determined by Fisher's method is given in table 7.

TABLE 7.—*Significance of variance between treatments of experiment 3*

	Treatments						Mean	Standard error
	Check (A + C)/2	B	D	E	F	G		
Per cent plants rotted <sup>a</sup> .....	380.8	126.1	196.3	420.9	164.5	226.8	270.8	26.77
Per cent of mean .....	140.6	46.6	72.5	155.4	60.8	83.8	100.0	10.67 <sup>b</sup>

<sup>a</sup> If all plants in any treatment were rotted the value would be 700, the sum of the 7 replicates.

<sup>b</sup> To be significant the differences between pairs of treatments must exceed  $2\sqrt{2 \times 10.67} = 30.17$ .

The effectiveness of treatments judged by Student's method (14) forms the same series as that by Fisher's method.

Note, in table 4, the spotted distribution of disease shown by the spread of the percentage of rotted plants in the plots of check A from 29.6 to 91.3 per cent. Plots of check C show a range from 47.6 to 64.9 per cent only.

Despite this demonstrated heterogeneity no statistically significant differences occurred between series A and C. This fact seems to justify the distribution of plots that was used.

Using the information in tables 5 and 7 as a basis, the treatments of experiment 3 may be arranged in a series ranging from the most effective to the least effective: (1) Bordeaux dip 1-0.7-3, (2) copper carbonate and lime dip 2-1-15, (3) Ferrox sulphur dust, (4) Ansul wettable sulphur dip 1-6, and (5) Bayer Special II-6 dip 1-3. The last-named treatment was without significant effect on the occurrence of heart rot.

*Experiment 4:* The experiment discussed below was installed on the island of Lanai during September, 1931. In the area selected for the test, 3 replantings had been necessitated by losses from heart rot. Approximately 40 per cent of these also had failed. The causal organism was found to be *Phytophthora parasitica*. The area used was a low one, subject to frequent inundation during heavy rains. It thus presented conditions of maximum severity for a control study. All plants from 4 beds 300 feet long were pulled up. The area was divided as follows: each of the 12 plots in the 2 outer rows was 25 feet long, while the 8 plots of each of the center rows were 36 feet long. There were 2 lots of planting materials: small slips cured 2 weeks, and similar slips cured many months. Some of each were used as nontreated checks, and equal numbers were dipped into freshly prepared Bordeaux 1-0.7-3. They were planted while wet. Thus there were 10 plots of each treatment distributed throughout the experimental area. This entire area was badly flooded by rains twice during the period of observations and more than 80 per cent of the nontreated plants rotted. In spite of these adverse conditions, the plants dipped in Bordeaux were surprisingly vigorous and the degree of control of heart rot was in excess of 70 per cent. Table 8 summarizes the conditions in this experiment after 6 months.

TABLE 8.—Summary of conditions in control experiment 4, after 6 months<sup>a</sup>

Treatment	Plants treated	Plants rotted	Control
		<i>Per cent</i>	<i>Per cent</i>
A R.C. Nontreated check <sup>b</sup> . . . . .	531	80.44	
B R.C. Bordeaux dip 1-0.7-3 . . . . .	578	19.97	74.95
C O.C. Nontreated check . . . . .	588	84.99	
D O.C. Bordeaux dip 1-0.7-3 . . . . .	575	24.78	69.65

<sup>a</sup> Odds indicative of the significance of differences between the treatments are: A-B, > 1 to 10,000; A-C, 1 to 2.75; A-D, > 1 to 10,000; B-C, > 1 to 10,000; B-D, 1 to 9.26; C-D, 1 to 10,000. To be significant odds should exceed 1 to 25.

<sup>b</sup> R.C. indicates recently cured slips; O.C., older cured slips.

The distribution of plots was such that statistical analysis could be obtained by using Student's method for paired observations (10). Results of this analysis (footnote a, table 8) demonstrate that there are no significant differences between slips of recent curing and slips of older curing, but that differences between any set of treated plots and nontreated plots are overwhelmingly significant.

*Experiment 5:* In a newly planted 200-acre field at Kemoo, Oahu, 10 per cent of the plants were estimated to have been killed by *Phytophthora parasitica*. Local spots of high incidence of disease were evident, where 50 to 75 per cent of plants in acre areas were killed. One such area, 50 beds wide and 300 feet long, was selected for the control test described below. Provision was made here for comparing (A), dipping slips in Bordeaux 1-0.7-3 and planting while wet, with (B), planting nontreated slips and immediately discharging enough of the same Bordeaux into their hearts to fill the plant and wet the soil thoroughly around the butt of each.

Seventy-five plots of 100 feet long and 2 beds (2 rows each) wide were laid out so that each line included a replication of each treatment. The distribution of plots was such that the 25 replications provided the following varieties and numbers of lateral contacts: A with B, 24; A with check 22; B with check 22. Not all plants in these plots were a part of the experiment. Certain of the original healthy plants were left standing. These were identified by painting a leaf of each with white paint. The test plants were used as replants and were placed where dead plants had been removed.

Table 9 shows the number of plants treated, the percentage of plants in

TABLE 9.—*A summary of conditions in control experiment 5, Kemoo, Oahu, after 6 months*

Treatment	Plants treated	Plants rotted	Control
		<i>Per cent</i>	<i>Per cent</i>
A Bordeaux 1-0.7-3 dip .....	3161	13.8 <sup>a</sup>	63.4
B Bordeaux liquid in hearts ....	3053	26.3	30.2
X Nontreated check .....	3082	37.7	

<sup>a</sup> Odds indicative of the significance of differences between the treatments are: A-B, > 1 to 10,000; X-A, > 1 to 10,000; X-B, 1 to 713.

each treatment that rotted, and the percentage control effected by the 2 types of applications. Added to this table as a footnote are the odds corresponding to the Z values of Student's tables (9). They show that all differences in table 9 are highly significant.

*Experiment 6:* The experiments already presented indicate that Bordeaux 1-0.7-3 is the most effective of all fungicides tested. A series of field studies was planned, therefore, to give information regarding the best means of applying it to planting materials. The first of these was located in an area where *Phytophthora parasitica* was abundantly distributed. Five means of applying Bordeaux 1-0.7-3 to slips were compared (Table 10).

The experimental area was laid out in 17 lines of increasing length. Each line, regardless of its length, was divided into 7 equal plots, 2 of which were used for checks, and 1 for each type of treatment; hence there were 17 replications of each treatment and 34 of the check. The plots in row 1 were each 13 feet long, in row 2, 15 feet long. They increased from row to row by an addition of 2 feet, so that plots of row 17 were 45 feet long. The distribution of treatments and check was random.

A very spotted development of disease occurred, the percentage of diseased plants in the nontreated checks varying from 3.4 to 78.7. Such uneven distribution of heart rot made small plots and many replications desirable.

Table 10 summarizes the pertinent data of experiment 6. It presents, for each treatment, the numbers of plants treated, the percentage of plants rotted, and the percentage of control effected. Footnote a of table 10 is

TABLE 10.—*Summary of experiment 6 after 6 months\**

Treatment	Plants treated	Plants rotted		Control
		Treated plots	Adjacent checks	
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
A. Bordeaux 1-0.7-3 dip in field	967	3.8	32.86	88.44
B. " liquid in hearts after planting	914	17.42	34.56	49.55
C. " butt spray, dry, top spray	932	12.18	31.75	61.64
D. " dip at trimming bed, plant dry	905	7.55	37.9	80.10
E. " dip as D, spray tops after planting	911	4.61	33.26	86.17
X. Nontreated checks	1892			

\* Odds indicative of the significance of differences between treatments are: A-X, > 1 to 10,000; B-X, > 1 to 10,000; C-X, > 1 to 10,000; D-X, > 1 to 10,000; E-X, > 1 to 10,000; A-B, > 1 to 10,000; A-C, > 1 to 10,000; A-D, 1 to 95; A-E, < 1 to 2; B-C, 1 to 20; B-D, > 1 to 10,000; B-E, 1 to 5,000; C-D, 1 to 600; C-E, 1 to 1,000; D-E, 1 to 155.

a brief summary of statistical analysis of the results prepared by Student's method for paired observations (10).

From table 10 it is apparent that the several methods of applying Bordeaux 1-0.7-3 may be arranged in the following series of decreasing efficiency: (1) treatment A, dipping in the field; (2) treatment E, dipping planting materials at the trimming bed and spraying tops after planting (table 10 shows no significant difference between treatments A and E); (3) treatment D, dipping at trimming bed and planting dry; (4) treatment C, spraying of both butts and tops; (5) treatment B, applying the liquid to the hearts of the planting materials after they have been planted.

*Experiments 7 and 8:* Another set of experiments to test various means of applying Bordeaux 1-0.7-3 was installed on Oahu as an integral part of field plantings. Two upland sites were selected in areas of excessively high rainfall, where *Phytophthora cinnamomi* caused heart rot in prior plantings. Rainfall in normal years was known to be in excess of 100 inches. During the first 6 months of the tests described below, 63.12 inches of precipitation occurred. During one 24-hour period 15.5 inches of rainfall, and during a second similar period 12 inches fell.

The probability that a single application of Bordeaux would provide enough fungicide to protect susceptible plants for a period of months under such conditions seemed slight. Provision was made, therefore, to determine the number of applications necessary to a high degree of control. Duplicate tests were installed at Opaepa and Waimea, Oahu. Bordeaux 1-0.7-3 was used according to 5 schedules (Tables 11 and 12).

TABLE 11.—Summary of control in experiment 7

Treatments	Plants treated	Plants rotted		Control	Odds <sup>a</sup>
		Treated plots	Adjacent checks		
		Per cent	Per cent	Per cent	
A. No dip; 3 liquid applications	1389	3.05	19.22	84.16	1249
B. Dip; 1 liquid application	1394	3.51	17.75	80.23	525
C. Dip; 2 liquid applications	1413	2.17	22.32	90.28	713
D. Dip butt only	1264	3.21	13.33	75.92	587
E. Dip entire plant only	1301	3.16	19.14	83.49	587
X. Nontreated check	4061				

<sup>a</sup> Odds are the values from Love's modification of Student's table (9) corresponding to the computed Z values.

The liquid applications were made from a portable spray tank, using no nozzle, (1) immediately after planting, (2) after 5 weeks, (3) after 4½ months—4 days after the 15-inch rain.

TABLE 12.—*Summary of control in experiment 8*

Treatments	Plants treated	Plants rotted		Control	Odds*
		Treated plots	Adjacent checks		
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
A. No dip; 3 liquid applications	1453	3.97	27.86	85.76	9,999
B. Dip; 1 liquid application	1345	3.37	32.40	89.60	4,999
C. Dip; 2 liquid applications	1331	2.28	23.08	90.13	> 10,000
D. Dip butt only	1426	5.90	24.72	76.14	3,332
E. Dip entire plant only	1356	2.75	21.07	86.95	> 10,000
X. Untreated check	4197				

\* Odds are the values from Love's modification of Student's table (9) corresponding to the computed Z values.

In both tests 8 replications of each treatment and 24 of the nontreated check were used. Each plot was 38 feet long and 4 rows (2 beds) wide. Each type of treatment was distributed throughout the experimental area and was in contact with nontreated check plots. Data from the 2 experiments are summarized in tables 11 and 12.

*Effect of Fungicides on Root Failure Caused by Heart-rotting Organisms:* *Phytophthora cinnamomi* is an active cause of root failure of pineapple plants as well as a cause of heart rot. Observations were made, therefore, to determine the action of fungicides, used for controlling heart rot, on the development of subsequent root-failure symptoms.

Plots of experiment 1, described above, were used for these observations. Five months after the plants had been treated all were examined and were grouped into 4 classes of varying vigor. The class divisions were arbitrary. Plants of class 4 had been removed from the field prior to the counts. Class 3 included plants that were yellow, stunted, and suffering from root failure. The very strong, prominently vigorous plants were tabulated as class 1, and all remaining plants, which were neither very good nor very poor, were placed in class 2. The figures thus obtained revealed a noticeable effect of the various fungicides upon the growth of the plants. All treated plots, regardless of the fungicides used, with the exception of copper carbonate dust, were better than nontreated plots. The indication is that, contrary to being detrimental to the growth of the host plant, the fungicides were beneficial. These observations are presented as table 13.

After 1 year, observations of the plots were again made. Root failure had caused the collapse of so many plants in all treated and nontreated plots alike that no counts were made. From the standpoint of the produc-

TABLE 13.—*Distribution of plants of various degrees of vigor in treated and check plots of experiment 1. Conditions after 5 months*

Fungicide	Percentage distribution			
	Class 1 Very good	Class 2 Fair	Class 3 Very poor	Class 4 Heart rot
Niagara colloidal sulphur . . . . .	78.89	18.27	2.54	0.3
Ferrox sulphur . . . . .	78.63	19.13	1.64	0.6
Fungi Bordeaux . . . . .	77.11	18.49	4.00	0.4
Lime sulphur . . . . .	63.12	26.55	8.43	1.9
Bordeaux 1-0.35-13.5 + resin . . . . .	61.48	28.60	9.92	3.8
Bordeaux 1-0.7-6 . . . . .	61.36	28.41	7.53	2.7
Copper carbonate 1-12 . . . . .	59.80	28.33	10.57	1.3
“ “ + lime 4-1-48 . . . . .	55.89	33.54	7.37	3.2
Bordeaux 1-0.7 -13.5 + resin . . . . .	55.89	26.72	11.79	5.6
“ “ no resin . . . . .	53.92	30.25	20.93	4.9
“ 1-0.35-13.5 “ “ . . . . .	53.23	26.69	13.38	6.7
Ialine sulphur 1-3 . . . . .	49.84	20.71	10.85	18.6
Copper carbonate + lime 4-1 (dust) . . . . .	48.19	42.00	9.81	
Bayer Special II-6 dust . . . . .	41.45	31.37	23.78	3.4
Check (slips) . . . . .	40.91	21.64	14.45	23.0
“ (crowns) . . . . .	29.00	20.00	3.40	47.6
Copper carbonate dust . . . . .	0.00	0.00	100.00	0.0

tion of marketable fruits, the entire experimental area was a loss. If such collapse were certain to occur in *Phytophthora cinnamomi* areas the use of any material to protect young plants from heart rot would not be justified. Experienced growers agree that while such losses may be anticipated in years of bad weather, they may be absent in good years. Clearly, the control of root rot caused by *Ph. cinnamomi* is a problem separate from the control of heart rot caused by the same organism. The use of Bordeaux dip in such areas is a matter to be determined from field records over a period of years. At present no such records are available. Plants protected from heart rot in *Ph. parasitica* areas mature fruit and are not subject to root failure of the type found in *Ph. cinnamomi* localities.

#### DISCUSSION

##### Plot Distribution and the Analysis of Data

To provide a fair test of the relative effectiveness of several competing fungicides for controlling soil-inhabiting organisms, such as the heart-rot pathogens, the plots of each treatment should be so distributed that the sum of the values of soil infestation for all replications be equal between treatments. Such an arrangement of plots was impossible in the present application owing to the absence of methods for measuring the distribution



of the pathogen. An effort was made, therefore, to so distribute plots that heterogeneity arising from sources other than the treatments themselves could be evaluated.

Experiments representative of several types of plot distribution are discussed above: Random distribution of one-line plots of each treatment and nontreated checks with few replications; random distribution of part-line plots in which the numbers of replications were increased; arrangement of treatments so as to insure many lateral contacts between the treated plots and between the treated and nontreated plots; and a modified Latin Square. Experiments of the first 2 types unfortunately did not provide an adequate number of replications in the one case or a proper distribution of checks in the other to make the results statistically significant. The results of experiments 1 and 2 must, therefore, be regarded as approximate only, but nevertheless indicative of major differences in the effectiveness of the several treatments.

The Latin Square permits a mathematical reduction of results in cases of regular soil heterogeneity (14). Experiment 3 of the present paper was laid out according to the requirements of the Latin Square, provision being made, however, for 2 sets of nontreated checks. Each set of checks was regarded as a separate treatment for the purposes of analysis by Student's method (14); hence a check was provided on the validity of applying the Latin Square to the class of irregular soil heterogeneity present in the experimental area. It does not follow, however, that equally significant results would be obtained from the Latin Square in all heart-rot areas. Provision must always be made for adequate checks of the installation, as a whole.

In an analysis of the Latin Square comparisons are made between groups of plots rather than between single plots, and any set of treatments is just as "close" to any other set as it is to the nontreated check. The opportunity for validly examining apparent differences between sets of treatments is thus enhanced. This method also permits a more adequate evaluation of heterogeneity from all causes beyond the treatments themselves than any other plot distribution known. These features make the Latin Square the desirable arrangement to be used. Where it cannot be used, the plots should be arranged for analysis by the method of paired observations.

#### Host Factors Influencing Control

In the experiments detailed above, indications of different susceptibilities of various classes of planting materials were evident.

A comparison of the relative susceptibilities of crowns and slips was made in experiment 1. Approximately 800 nontreated crowns were dis-

tributed in 10 equal plots throughout the experimental area. Ten similar areas of nontreated slips were contiguous with the narrow dimension of the plots of crowns. Other plots of slips were adjacent to the long dimension of the areas. In all, 16 plots of slips were located for comparison with the 10 plots of crowns. When changes no longer occurred, 19.39 per cent of the slips and 55.54 per cent of the crowns were diseased. Only 35.5 per cent as much rot developed in the slips as in the crowns. It is clear from these figures, and from other observations not presented, that nontreated crowns should not be used where heart rot occurs.

Preliminary indications from experiment 2 were that nonstripped crowns were less susceptible to heart rot than stripped. Control effected by treated crowns of the former type was 84.4 per cent contrasted with 75.6 per cent for treated crowns of the latter type. While these differences are not statistically significant, they agree with greater differences noticeable in nontreated crowns of the 2 types shown in plantation experiments not reported here. Data regarding the effect of stripping on the susceptibilities of slips or shoots to heart rot are not now available.

#### Control Effected by Sulphur Preparations:

Experiments 1, 2 and 3, detailed above, incorporated the use of Ferrox sulphur, Niagara colloidal sulphur, Ansul wettable sulphur, and Ialine wettable sulphur. A great spread in the effectiveness of the sulphur fungicides in the different experiments is noticeable. In experiment 1 Ferrox sulphur and Niagara sulphur gave a control of 97.13 per cent and 97.18 per cent, respectively. In all subsequent tests sulphurs exhibited little efficiency as agents in the control of heart rot. Thus in experiment 2 the control was -8.19 per cent for Ferrox sulphur and -11.78 for Niagara sulphur; in experiment 3, 48.53 per cent for Ferrox sulphur and 40.45 for Ansul wettable sulphur.

Experiments 1 and 3 were in areas where *Phytophthora cinnamomi* was the pathogen; experiment 2 where *Ph. parasitica* was the pathogen. No explanation can be made at this time to account for the demonstrated differences in effectiveness of the sulphurs in the above experiments. It is interesting to note that less than two grams of the fungicidal dusts were added to each plant in experiment 1, where control was 97 per cent. At the site of experiment 2 an application of 800 pounds of wettable sulphur per acre to the soil, an equivalent of 36 grams per plant, gave only 30.3 per cent control.

There is no apparent correlation between the quantity of rainfall and control in the several areas. Hydrogen-ion and temperature data are not available for all the locations.

### Control Effected by Bordeaux Dip:

Bordeaux dip was the one treatment used in all the experiments reported above. Figure 4 graphically presents for each experiment the contrasts existing between the percentage of plants rotted in plots treated with Bordeaux dip and in contiguous nontreated plots. It shows strikingly the beneficial effect of the fungicide. It also emphasizes the variability in the degree of control experienced in the several experiments.

The areas in which the above tests were installed were selected because they represented extremes in conditions favoring heart rot. The control experienced in them is even less, therefore, than may be anticipated in areas of average losses.

Bordeaux, used as a dip, has been more desirable than any other treatment used to control heart rot in the present studies. It combines the qualities of effective control, ease of application, low cost, and absence of toxicity to the pineapple plant. As seen in table 1, wide fluctuations in the ratio of lime to copper sulphate, and in the concentration of the Bordeaux are possible without ill effects to the plants treated. This feature makes Bordeaux especially desirable for use in the fields, where accurate weighing of the ingredients is not always possible.

### Control Effected by Copper Carbonate:

Copper carbonate, although effective in controlling heart rot, causes undesirable burning of the plants treated. In experiment 1, detailed above, many of the plants treated with copper carbonate as a dust or as an aqueous suspension failed to take root or to grow even after one year. Those that did grow were smaller and less vigorous than plants in adjacent plots treated with other preparations. Burn appeared at all points where the plants were injured.

Limited tests have shown that the burn from copper carbonate can be largely eliminated by the addition of hydrated lime in the ratio of 4 parts of carbonate to 1 of lime.

### Dusts Compared with Liquid Fungicides:

Under the conditions of tests to date, liquid fungicides seem more desirable than dusts. In windy regions appreciable quantities of the dusts are soon removed. Wet planting material cannot be used safely with dust applications because of the caking of destructive quantities of the materials upon it. Liquid preparations, in contrast, have been very lasting in windy areas and in the regions of high rainfall. No appreciable difference has been noted in burning or adherence of liquid treatments when dry or slightly wet planting materials were used.

### Dipping Compared with Other Methods of Applying Liquid Fungicides:

From the experiments detailed above, it is evident that a single application of Bordeaux 1-0.7-3, if applied by completely immersing the planting material in the fungicide, protects susceptible plants better than larger amounts of the same or different fungicides applied in other ways.

In experiment 5, reported above, a comparison was made of applying Bordeaux, as a liquid, into the hearts of newly set plants with dipping planting materials in the same material before planting. Although 3 times as much fungicide was required to treat the plants by filling the hearts with liquid as by dipping before planting, the control experienced by the former treatment was less than 50 per cent of that effected by the latter.

From table 10, which summarizes the pertinent data from experiment 6, it is evident that Bordeaux applied as a dip is more effective than Bordeaux of the same strength applied in other ways. Experience gained during the installation of this experiment and of the 2 Oahu tests, discussed above, recommends dipping of the whole plant, for 2 reasons: A higher degree of control is obtained, and less labor is required than for dipping only the butts of plants. There is no appreciable difference between the incidence of disease in plots of slips planted while the fungicide was wet and in plots planted after the Bordeaux had dried. The indications are that dipping may be done at the trimming bed if treatment there is thorough; but, because of the extra care required to assure success when treatments are applied at any considerable distance from the field, it is recommended that dipping be done as near the place of planting as possible.

In experiments 7 and 8, treatment A provided for 3 liquid applications, spaced over a period of weeks, to the hearts of newly set plants. For treatment A nearly 9 times as much fungicide was required as for treatment E, the standard complete dip, yet the latter treatment was as effective in controlling heart rot as the former. Obviously, dipping is the desirable method to use.

The use of stickers did not appear to alter significantly the percentage control by fungicides with which they were used, but did increase their toxicity to the host plant. Their use is not recommended.

The significance of different methods of applying the fungicide for the control of heart rot in relation to the epidemiology of the disease is considered elsewhere (12).

### Control Recommendation:

For the control of *Phytophthora* heart rot of the pineapple the use of a Bordeaux dip 1-0.7-3 is recommended. Only the most vigorous slips of recent curing should be treated. These should be wholly immersed in the

freshly prepared fungicide, preferably in the field where they are to be planted. They may be planted either before or shortly after the fungicide has dried upon them. If slips are not available, crowns may be used. Well cured, nonstripped crowns have given the best control for this class of planting materials. Their use is not unreservedly recommended, however, because of their greater susceptibility to yellow spot, a virosis, and possibly to other diseases.

Records kept for the experiments described in this paper show that the materials required to treat 3,000 slips cost \$2.00 or approximately 0.07 of a cent per slip. Crowns can be treated for less.

The fungicide used in these studies, prepared according to the procedure given below, was selected after preliminary studies only. It is recognized that additional investigation of means of preparing the Bordeaux may increase the degree of control obtained. To date, the best preparation has resulted from the use of crystalline copper sulphate and fresh Waianae hydrated lime. The copper sulphate was dissolved in one-half the quantity of water required for the complete fungicide. Shortly before the Bordeaux was to be used, the hydrated lime was thoroughly mixed with the remaining water in a separate container. While stirring the copper sulphate solution the lime suspension was poured into it. The mixture was thoroughly stirred both before and during treatment of the pineapple planting materials. The above procedure for preparing the fungicide is recommended until better methods have been defined.

Since the incidence of heart rot is dependent upon the environment as well as upon other factors listed in an earlier part of this paper, original plantings should be treated only in areas where repeated losses in successive plantings have been demonstrated. In the absence of definite information covering a number of years, and under ordinary field conditions, only replants should be dipped in Bordeaux.

Heart rot was segregated above into 2 classes, that occurring in very young plants soon after planting, and that occurring in more mature plants. The control measures discussed in this paper are planned primarily as a means of limiting the loss of replants resulting from heart rot of the first and more usual class. Means of controlling losses from the second class of disease remain to be studied.

#### SUMMARY

Heart rot of the pineapple plant in Hawaii is caused by three species of Phytophthora: *Ph. cinnamomi*, *Ph. parasitica* and *Ph. palmivora*. Experiments in naturally infested areas involving 2 of these fungi have shown that Bordeaux 1-0.7-3 as a dip is an effective and economical control. This formula was selected from 67 formulae of 22 liquid and 12 dry fungicides

tried. Bordeaux 1-0.7-3, applied by completely immersing planting material in it has given better control than larger quantities of the same or different fungicides applied in other ways. A single application in 8 separate experiments has given the following control under conditions extremely favorable to the development of the disease: Average control 80.29 per cent, range 63.40-90.95 per cent; disease in adjacent nontreated plots, average 44.8 per cent, range 19.14-83.07 per cent: The total concentration of Bordeaux may be doubled, or either constituent varied 50 per cent without injuring plants treated. The cost of materials is approximately \$7.00 per acre of 10,000 plants. Specific recommendations for the use of this treatment have been made.

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# SNOWMOLD OF TURF GRASSES AS CAUSED BY *FUSARIUM NIVALE*<sup>1</sup>

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## INTRODUCTION

Winterkilling of turf grasses presents an important problem in the maintenance of fine turf, particularly in the northern States. Winterkilling is a general term that has been used in referring to injuries to turf caused by poor drainage, to drying of grass due to cold dry winds, late freezes after the grass has begun to grow in the spring, and to fungous disease. The disease, of parasitic origin, occurs during the winter and early spring and is called snowmold because of its association with snow. The purpose of this study was to determine the cause of this disease and investigate the relation between the host and the parasite. It was also desired to determine the climatic and soil conditions that favored its development, influences of cultural practices, and to work out some means of control.

## GEOGRAPHIC DISTRIBUTION AND HOST RANGE

Snowmold is prevalent in Europe, where it occurs on winter grains and lawns in Germany, Denmark, Norway, Sweden, and Russia. The destruction of grain was recorded by Knutberg (7) in 1768. Some of the early studies of the disease and its causal organism were made by Fries (2), Unger (19), Pokorny (13), Fuckel (3), and Sorauer (15, 16, 17).

In North America it is prevalent on golf courses and lawns in the northern tier of States and in Canada. It is most important in Wisconsin, Minnesota, Michigan, and Canada, but also occurs with severity in other States in the same latitude. In the States south of the northern tier it is frequently found in a few places but causes less damage. It has been reported as far south as Virginia.

## DESCRIPTION OF THE DISEASE

Most of the damage to turf in this country occurs on putting greens of golf courses, but it also occurs on fairways and lawns. When it occurs on

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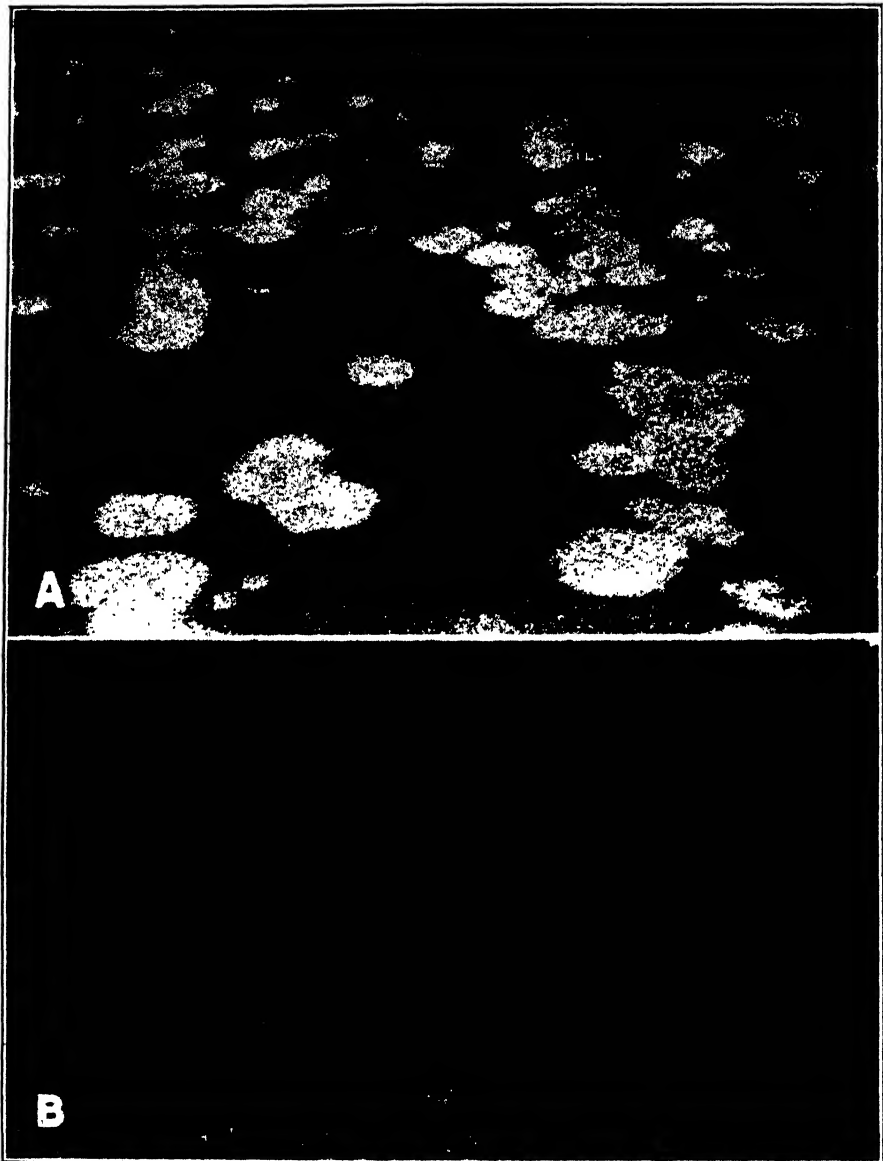


FIG. 1. A. Damage to green at Grand Rapids, Mich., caused by *Fusarium nivale*. The lighter areas are snowmold patches. Photographed on April 3, 1928. The grass on this green did not recover fully until midsummer. B. Plots of putting-green turf showing control of the disease with corrosive sublimate. The treated plot was laid out in the shape of an L and the diseased area on the left and in the background was nontreated. The fungicide was applied in the fall and the photograph was taken in April of the following year.



putting greens it may kill the turf outright; but, even when the disease is not serious enough to kill the grass, it often takes a long time for the grass to recover. Weeds are apt to develop in the weakened patches because of the absence of competition from the grass.

The disease is noticeable as soon as the snow melts, when dead areas are found in the turf. These areas usually are irregularly circular, from a few inches to a foot or more in diameter and, in severe cases, may run together and cover large areas (Fig. 1, A). The patches usually are whitish gray, the leaves have a bleached appearance and, when wet, feel slimy. These patches develop under the snow while it is melting and at that stage they are often covered with an aerial mycelium (Fig. 2). When the conditions remain long favorable for its development, the aerial mycelium may be so abundant that the leaves are matted together, forming a thick layer

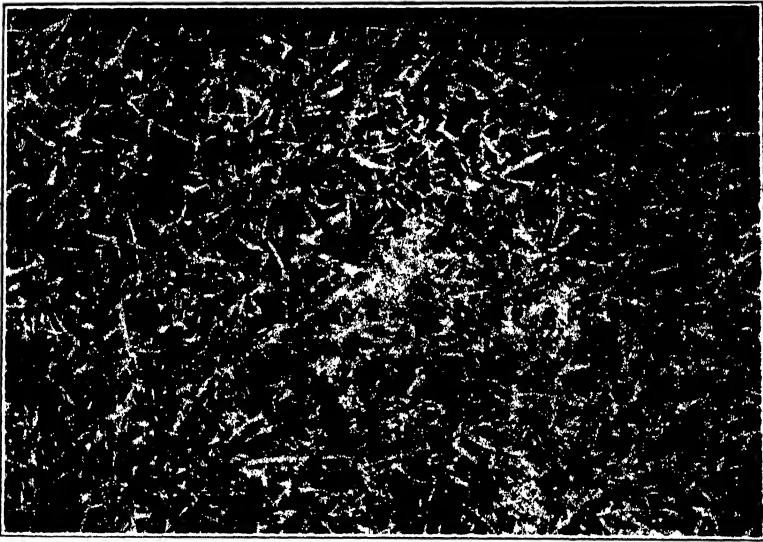


FIG. 2. Fluffy mycelium of the fungus growing over the leaves in the first stage of a snowmold patch.

over the patch. Under this layer the fungus may continue to develop after the grass has dried and the turf may be killed. When the patches are exposed to the sunlight a pinkish cast often becomes apparent on the leaves, due to a change in color of the mycelium. Light brown areas of turf killed by other agents are readily distinguished from snowmold injury. Patches not having an abundant growth of mycelium are frequently not killed but will recover slowly. The organism usually attacks only the leaves, but when conditions are favorable for a long period it spreads into the stems and crowns.

The disease occurs on Kentucky bluegrass, *Poa pratensis* L., annual bluegrass, *P. annua* L., redtop, *Agrostis alba* L., creeping bent, *A. palustris* L., velvet bent, *A. canina* L., Colonial bent, *A. tenuis* Sibth., red fescue, *Festuca rubra* L., rye, *Secale* spp., wheat, *Triticum* spp., and barley, *Hordeum* spp.

#### CAUSAL ORGANISM

Snowmold occurring on winter grains in Europe has been shown to be due mostly to *Fusarium nivale* (Fr.) Ces. (*Calonectria graminicola* (Berk. and Brm.) Wr.) and in part to *F. culmorum* (W. G. Sm.) Sacc. Schaffnit (14) in examining material from the field found *F. nivale* in 28 out of 29 samples and *F. culmorum* in the one exception. Lindfors (8) examined at least 200 samples and found *F. minimum* (*nivale*) present in all of them. Schaffnit (14) was able to produce snowmold-like symptoms by inoculating with *F. culmorum*, *F. herbarum* (Corda) Fries, *F. didymum* Harting, *F. avenaceum* (Fr.) Sacc. and *F. lolii* Smith as well as *F. nivale* on etiolated or weakened plants. Lindfors (8) believed that *F. minimum* (*nivale*) was the most important and that the others may play a minor part in the occurrence of the disease. Atanasoff (1) inoculated pots with *Gibberella saubinetii* (Mont.) Sacc., *F. culmorum*, *F. culmorum* (W. G. Sm.) Sacc. var. *leteius* Sherb., *F. avenaceum* (Fr.) Sacc., *Helminthosporium sorokinianum* Sacc. and two other undetermined *Helminthosporium* spp. and produced conditions similar to those occurring in the field but apparently he did not make his inoculations when the temperature was near the freezing-point, the optimum for true snowmold.

Numerous inoculations have been made in moist chambers maintained at temperatures between 0° and 5° C. with *Fusarium nivale*, *F. culmorum*, *F. avenaceum*, *F. solani* Ren. and Bert., *Gibberella saubinetii*, and numerous other undetermined fungi isolated from diseased grass leaves. Of all these species only *F. nivale* proved pathogenic. Other soil fungi are often found associated with *F. nivale* in diseased patches of turf and are, at times, more conspicuous than the primary organism. In pure-culture inoculation, however, these do not produce the disease.

Cultures of *Fusarium nivale* that were used in inoculation work were isolated from diseased leaves of grass taken from patches showing symptoms of snowmold. The diseased leaves were placed in Petri dishes on water agar and transfers were made from the colonies that developed. The cultures were then grown on potato-dextrose agar. Isolations were made with material from Madison, Wisconsin, Grand Rapids, Michigan, Chicago, Illinois, and from Minneapolis and Duluth, Minnesota. Single-spore cultures were made by the dilution method after the cultures began to sporulate.

*Cultural Studies:* The cultures were grown on corn-meal agar, oat-meal agar, potato-dextrose agar, and grass clippings. On corn-meal agar there was a scant aerial, colorless, and sterile mycelial growth. A very abundant mycelial growth developed on oat-meal agar and spores were produced in slimy salmon-color masses, often covering a large part of the surface of the agar. On potato-dextrose agar there was an abundant, white, fluffy, spore-bearing mycelium. On sterile grass clippings, the fungus covered each leaf with a fluffy white mycelium that in a few cases produced spores.

When the cultures were kept in darkness from the time they were isolated, there was no spore production, but when transferred to diffuse light, especially in a moist chamber, sporodochia were formed and great masses of spores developed. After the spores were once produced in light, the cultures continued to sporulate on subsequent cultures, when transfers of spores were made. Light also changes the color of the mycelium from white to a pale pink, which was demonstrated in Petri-dish and test-tube cultures and on mycelium on potted grass plants.

Schaffnit (14) found that *Fusarium nivale* grew between temperatures from 0° to 30° C., with an optimum of 22° C. Lindfors (9) and Tu (18) also found that the optimum temperature was 22° C. In the studies herein reported 3 cultures of *F. nivale*, isolated from diseased leaves from snow-mold patches, were grown in constant-temperature chambers from 2 to 36° C. The cultures were grown in Petri dishes containing 10 cc. of potato-dextrose agar. Transfers of mycelium were made from young Petri-dish cultures, and the hourly rate of growth was calculated after 72 hours of growth.

Figure 3 shows the hourly growth rates at the various temperatures. The 3 cultures agree generally in their growth curve. The amount of growth at 2° C. indicates that the fungus is adapted to low temperatures. In general, the optimum appears to be about 20° C. and all of them grow as well or better at 16° as at 24°. There is a sharp drop in the curve above 24° and, although there was some growth at 32°, there was none at 36°.

*Infection Experiments:* The pathogenicity of the organism was proved by inoculation of potted grass plants. All of the grasses were grown in autoclaved pots and soil. The seed was treated with 1-320 Formalin for 15 minutes. The grasses were grown in the greenhouse and clipped from time to time, so that the plants were usually about 2 inches high.

The strains of the fungus were grown on sterilized grass clippings in culture tubes until the mycelium had grown throughout the clippings in the tube. A portion of the clippings was then transferred to the center of a pot of grass and held about 1½ inches above the surface of the soil by the leaves. The pots were then placed in moist chambers. Most of the inoculations were placed in a chamber kept from 0° to 5° C., since best results

were obtained at that temperature. Successful inoculations were made on creeping bent, redbtop, Kentucky bluegrass, fescue, barley, wheat, rye, and oats. All of the inoculations were made on seedlings, except creeping bent, where old sod was brought into the greenhouse and potted.

In most cases infection of the leaves had taken place within 2 days after inoculation. When the amount of moisture in the chamber was decreased the infection was slight and the spread of the organism from leaf to leaf was slow. The mycelium of the organism spread quickly, when the atmosphere of the chamber was foggy, until all the leaves of the grass were covered with mycelium, as in figure 4.

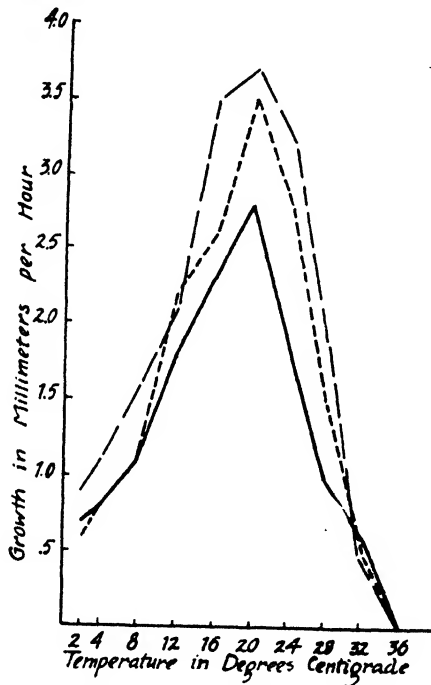


FIG. 3. Effect of temperature on the rate of growth of 3 strains of *Fusarium nivale* on potato-dextrose agar. Note their essential likeness with a rather slow rise up to 10°, then more rapid up to the optimum of about 20°, with a rapid fall above 24°. This is in contrast with the most culturable parasitic fungi, which show an optimum at about 24–30° C.

Inoculations were placed in chambers kept at temperatures from 0–5°, 5–10°, 10–15° and 15–20° C. The best results were obtained in the 0–5° chamber. The mycelium spread quickly and was abundant and fluffy. In the 5–10° chamber there was also an abundant growth of mycelium and a rapid spread of the fungus over the leaves. At 10–15° the development was much slower and the mycelium was less abundant. At 15 to 20° there

was a very small amount of infection near the inoculum; there was no aerial mycelium and, after 3 weeks, very slight spread of the organism.

*Histological Studies:* Material obtained from plants of Kentucky bluegrass, redtop, and creeping bent, inoculated with pure cultures in the greenhouse, was used to determine the method of penetration of the organism and the relation of the fungus and host tissue. Upon examination of prepared slides, it was found that the mycelium developed along the outside of the leaf and, when a hypha reached a stoma, it enlarged at the terminal end and one or more infecting hyphae developed down through the stomatal opening into the intercellular spaces. (Fig. 5, A). No direct penetration through the epidermal cells was observed until after the cells within the leaf had collapsed and there was an abundant development of mycelium. The

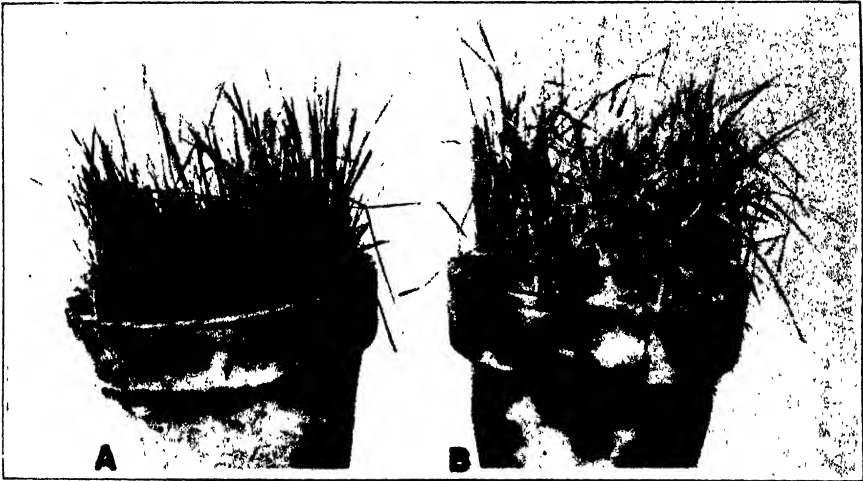


FIG. 4. Inoculation of pot culture of Kentucky bluegrass with *Fusarium nivale*. A. Control. B. Diseased plants with an abundance of aerial mycelium.

mycelium developed faster along the surface of the leaf than it did within the leaf tissue, so that new infections through stomata took place along the entire length of the leaf until it became wholly infected. When temperature and moisture conditions were not favorable for abundant aerial mycelium, the infection of the leaf was slower because less mycelium grew along the surface.

The growth of the organism in the tissue is intercellular with much branching until numerous hyphae extend in all directions. Later the cells collapse and the fungus almost fills the space occupied by the collapsed cells. The fungus then penetrates into the vessels.

It was observed that when a leaf is badly diseased, sporodochia develop through the stomata in rows. An abundant growth of mycelium at first

occurs through the stomata and later develops into sporodochia that bear great masses of spores on club-shape sporophores (Fig. 5, B). Most of the spores become detached during the preparation of the slides. In some



FIG. 5. A. Infection of a leaf through a stoma. Note the enlargement of the end of the infecting hypha and the intercellular course of the invading hyphae. B. Sporodochium as it occurs on a stoma. The guard cell of the stoma is located just below the sporodochium.

cases the sporodochia become so large that they cover a considerable surface of the leaf, so that 2 or 3 stomata are involved. After the leaf is badly

diseased, penetration through the epidermis takes place in great abundance, nearly all of the parenchyma cells collapse and only the vessels are left intact.

#### INFLUENCE OF WEATHER ON OCCURRENCE OF DISEASE

Weather conditions play an important part in the development of the disease. Unger (19) reported that snowmold was serious after a wet fall, when snow fell on unfrozen ground. Pokorny (13) also found snowmold very severe on lawns during the spring of 1865, after a winter of continuous snow cover and a slow thaw in the spring.

Sorauer (15, 16) stated that the organism grew continually during mild winters and stopped only when it freezes. He explained that cavities were formed between the snow cover and the ground and in these cavities there was a saturated atmosphere and low temperature, which was suitable for the development of the fungus.

Hiltner and Ihssen (6) found snowmold worse on northerly slopes where the snow melted slowly in the spring. Mortenson (12) observed that the disease was worse after wet summers, when the moisture in the ground was abundant as the snow fell.

In Minneapolis, Minn., in the fall of 1927, when snow fell before the ground was frozen, severe attacks of snowmold were found on some of the putting greens the next spring. The same was true that year in Grand Rapids, Mich., where the snow melted before the end of the year and the disease was apparent in the latter part of December.

Deep snow, however, is not always necessary for the development of the disease. During the winter of 1927-28 at Madison, Wis., many greens on some of the courses were bare much of the time and snowmold was present on these greens. There was much cold and cloudy weather, and apparently there was sufficient soil moisture and atmospheric humidity to promote growth of the organism.

Sunlight and dry winds soon stop the development of the organism after the snow has disappeared. Where there has been an abundant growth of mycelium and the leaves are matted together, air and sunlight are kept out, moisture is held in the turf, and the organism continues to grow and to injure the turf severely. Hiltner and Ihssen (6), Atanasoff (1), and Schaffnit (14) record that the mycelium disappears as soon as the sunlight or air currents come in contact with it and the plants then begin to recover. In turf, where the plants are crowded, sunlight does not penetrate and the fungus may continue to live. Whenever a cloudy day occurs, it may again become active and spread. Greenkeepers have made a practice of brushing these patches to break the mat of leaves and mycelium. This dries the patches and lets the sunlight in, so that the development of the organism is stopped.

During the winter of 1928-29 temperatures were recorded under the snow at Wayzata, Minn. The thermograph bulb was placed under a covering of straw and during the winter was buried under a thick layer of snow. The temperature remained almost constant at 32° F., although the air temperature was as low as -20° F. In the winter of 1930-31 soil temperatures were recorded at Madison, Wis., where the thermograph bulb was placed just beneath the surface of the turf. When snow was present, the temperature remained nearly constant at 0° C. no matter what the air temperature was. When the snow melted, the soil temperature began to vary with the air temperature. The temperature under a covering of straw was the same as in the uncovered plots when snow was present, so that the covering of straw evidently did not raise the temperature. These temperature records indicate that the development of the organism takes place at about 0° C. This has been borne out by inoculation in the greenhouse where best results were obtained from 0° to 5° C.

#### INFLUENCE OF SOIL AND CONDITION OF GRASS ON OCCURRENCE OF DISEASE

Condition of the soil may affect the occurrence of the disease in two ways, first, by its influence on the growth of the plants and second, by its influence on the development of the organism. Hiltner (4) found the disease worse on heavy, poorly worked, poorly drained, clay soil, and on soil rich in humus. Hiltner (5) also found that manuring and covering with straw encouraged the development of the organism.

In rich soils there is a very luxuriant growth of the plants, late in the fall, just before the snow falls; and when the grass is fertilized late there also is a marked growth of grass. Plots of putting-green grass fertilized with ammonium sulphate on November 6, 1930, in Chicago, Ill., when observed on December 6, had a very dark green appearance in contrast to the checks, which had not been fertilized and were decidedly brown. Even though the temperature be very low, grass will grow when stimulated by fertilizer and the development of snowmold may be encouraged.

Soraauer (16-17) states that the fungus attacks the leaves mainly because of its thinner epidermis and its rich plasma. Hiltner and Ihssen (6) found that snowmold was worse on fields where the plants were larger and held the snow up from the ground, so that cavities were formed where the fungus could develop. They also found that grazing in the fall reduced the amount of snowmold because of the smaller amount of leaf surface present when the snow fell. Lindfors (8) recommended late seeding, low rates of seeding, and cutting or grazing in the fall, so that the leaf area would not be too great. He also states that when a mat of leaves and mycelium are formed, under which the fungus may continue its development, the plants are severely injured.



It has been the practice of some greenkeepers, especially in the vicinity of Minneapolis, to cover their greens with straw during the winter. In many cases this practice has led to very serious damage from snowmold. Other greenkeepers who fertilize in the fall, or have a great deal of organic matter in their top dressing, have trouble with serious attacks of the disease.

In order to determine what effect fall fertilization or straw covering would have on disease incidence, experiments were made on turf of putting greens during the winters of 1928-29 and 1929-30.

In the fall of 1928 plots of turf were laid out on a green of a golf course near Minneapolis. All combinations of corrosive sublimate, fertilizer, and straw were applied so that the experiment included plots treated with corrosive sublimate; corrosive sublimate and straw; corrosive sublimate and fertilizer, corrosive sublimate, fertilizer, and straw; fertilizer with and without straw; and straw and check plots. The use of corrosive sublimate and calomel as fungicides will be considered farther on.

The corrosive sublimate, at the rate of 3 oz. to 1000 sq. ft., was diluted with a quart of sand and broadcast over the plots. The fertilizers used were activated sludge, manufactured by the Milwaukee Sewage Disposal Commission, and ammonium sulphate. These were applied at the rate of 28 lbs. of sludge and 6½ lbs. of ammonium sulphate to 1000 sq. ft. and were also broadcast on the plots. The straw was applied Dec. 1 in a layer about 2 in. thick.

Table 1 gives the results of this experiment, the figures representing the percentage of the area killed by the disease. These percentages were determined in the spring after the straw had been removed on April 3. From

TABLE 1.—*Results of field experiments on plots of putting-green turf. The figures represent the percentage of turf affected by snowmold*

Materials applied	Minneapolis		Grand Rapids	
	Covered with straw	Not covered	Covered with straw	Not covered
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Checks . . . . .	100	0	50	20
Corrosive sublimate . . . . .	5	0	20	2
Corrosive sublimate and fertilizer . . . . .	50	0	30	10
Fertilizer . . . . .	100	0	90	40
Calomel and fertilizer . . . . .			70	20
Calomel . . . . .			30	5

the table it will be noted that when no straw had been applied there was no disease and where straw was applied alone, the grass was completely killed by snowmold (Fig. 6, B). The grass was killed also where the straw and the fertilizer had both been applied. Where corrosive sublimate had been applied only 5 per cent of the area of the turf was injured by disease. In

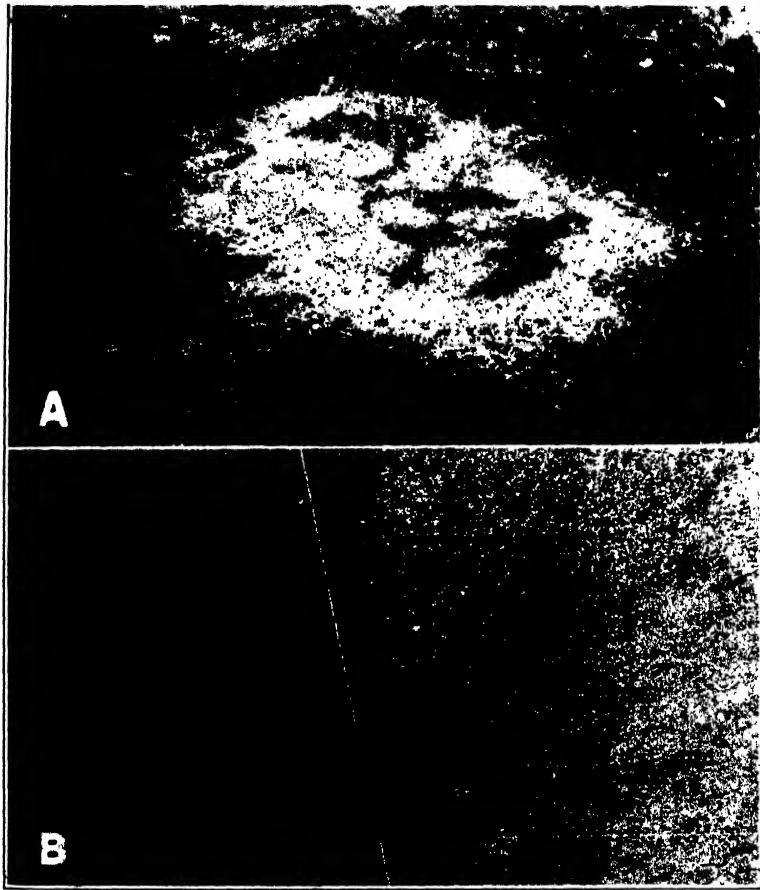


FIG. 6. A. Patch of wild creeping bent with two strains mixed together, one susceptible and the other resistant. The lighter area in the patch is the susceptible strain diseased with snowmold and the darker area is the resistant strain with no disease. B. The turf on the right of line was covered with straw during winter. The lighter area is the dead grass killed by snowmold. The darker area on the left is healthy green grass.

the plots where corrosive sublimate and fertilizer had been applied and covered with straw, 50 per cent of the turf was killed.

This experiment was repeated in the winter of 1929-30 on a golf course near Grand Rapids, Mich. All combinations of corrosive sublimate, fer-

tizer, and straw also were applied to these plots, and one series was made where calomel was substituted for corrosive sublimate. The corrosive sublimate and calomel were applied at the same rate as in the previous experiment. The fertilizers, activated sludge, and ammonium sulphate, were applied at the rates of 25 lbs. of sludge and 5 lbs. ammonium sulphate to 1000 sq. ft.

The results of this experiment, as given in table 1, show that the efficiency of control by chemicals is greatly reduced by fertilizers and straw covering. There was a more general distribution of disease over all of these plots but none of them suffered so severely as some of the areas of the previous year's experiment. The most severely injured plot was that fertilized and covered with straw, 90 per cent of which was covered with disease. In every case the covering of straw more than doubled the amount of disease and in some cases the disease was increased many times. Fertilizing also more than doubled the amount of disease. Not only do straw covering and applications of fertilizer in the fall increase the amount of disease, but it is much more severe; more of the stolons are infested and the turf recovers more slowly than when the disease occurs where the turf was not fertilized or covered with straw. The results of these experiments show the efficiency of mercury fungicides when applied alone and that this effectiveness may be much reduced by cultural practices.

#### FIELD EXPERIMENTS ON CONTROL MEASURES

Diseases of grasses in turf have been controlled for the last few years by broadcasting or spraying fungicides on the turf. Monteith (11) was able to control brownpatch, caused by *Rhizoctonia solani* Kühn, and dollar-spot caused by *R. sp.*, by treating the turf with mercury compounds. The most practical of these have been corrosive sublimate and calomel. For this reason, when a control for snowmold was sought, attention was turned to these chemicals.

Monteith (10) made the first preliminary tests during the winter of 1926-27, when he treated plots of turf on greens of a golf course at Madison, Wis., with corrosive sublimate at rates of 1 and 2 oz. to 1000 sq. ft. These treatments were effective in preventing snowmold in the spring of 1927.

Since then, experiments have been conducted to determine the amount of fungicide necessary to the effective control of the disease. In these experiments, plots of turf were laid out on greens of golf courses at Grand Rapids and Minneapolis. These plots were treated at the rates of 1, 2 and 3 oz. to 1000 sq. ft. The plots were separated by 2-ft. strips of nontreated turf to serve as checks. The chemicals were applied by carefully mixing each with one-half bucket of sand or fine compost and this mixture was broadcast over the plot. The checks were treated with the same amount of sand or compost without the fungicide.

Table 2 shows the results obtained on 4 of the experimental plots. The figures represent percentages of area of the turf affected by disease. The largest rate of application was not entirely effective in most cases. However, where the disease did occur on these plots and where it occurred on greens treated with a 3 oz. to 1000 sq. ft. rate, it was rarely severe and the turf soon recovered. The 2-oz. rate decreased the amount of the disease considerably, and here, too, the severity of the injury was lessened. The 1-oz. rate was not very effective, although it did lessen the severity of the disease to some extent. In the checks the amount of disease was not only greater but the injury to the grass was severe and the turf took longer to recover. Figure 1, B illustrates the effectiveness of control of snowmold with corrosive sublimate.

Golf courses that had been troubled with snowmold for several years have for the past few years been making a practice of treating all of their greens with either calomel or corrosive sublimate and have been successful in preventing serious attacks. Calomel and corrosive sublimate are almost equally as effective and both are used widely by greenkeepers. Some greenkeepers treat only those greens which are likely to be attacked and thus are saved the expense of treating all of their greens.

TABLE 2.—*Results of field experiments on control of snowmold on putting greens on golf courses. The figures represent the percentage of diseased turf. Each series of plots was laid out on different greens*

Series number	Grand Rapids		Minneapolis	
	1	2	3	4
Treatments	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
3 oz. Corrosive sublimate	5	5	0	5
3 oz. Calomel	5	20	0	5
2 oz. Corrosive sublimate	40	40	0	20
2 oz. Calomel	20	40	0	20
1 oz. Corrosive sublimate	80	60	20	40
1 oz. Calomel	40	20	40	20
Checks	90	90	80	60

#### VARIATION OF RESISTANCE TO DISEASE IN STRAINS OF GRASS

It has been observed that there is a difference in the susceptibility of varieties of winter grains, to attacks of *Fusarium* blight. Hiltner and Ihssen (6), Lindfors (8), and Atanasoff (1) mention this difference in resistance. The same has been observed to be true with different grasses used on golf courses.

The amount of snowmold that may occur on a golf course may be governed by several factors. It may be due to the location of the greens, that

is whether or not they are sheltered from sun and wind and are covered with deep drifts for long periods in the winter. On such greens the snow melts slowly in the spring and poor drainage may make attacks of the fungus more severe. The methods of culture used by the greenkeeper is another factor. If he fertilizes heavily late in the fall or supplies an abundance of organic matter, it is probable that he will be troubled with attacks of snowmold.

The resistance of the grass to snowmold also is a factor in the occurrence of disease on any particular golf course. It has been observed that some strains of creeping bent are very susceptible, while others are noticeably resistant. The Columbia type strains, which make a thin turf, are often severely damaged by numerous patches of snowmold. The turf does not recover in these patches, but must fill in from the edges of the patches when growing weather comes. This type of grass is used on several golf courses in Grand Rapids and Minneapolis, and is one of the reasons why those particular courses are troubled with snowmold. The Washington strain of creeping bent is often attacked by snowmold, but the disease is rarely serious enough to warrant resodding and usually the injury disappears early in the spring. Seaside creeping bent, which is propagated by seed, while the others are propagated by stolons, also is a susceptible strain, and when observed on golf courses it usually is severely attacked by disease. On demonstration plots in Detroit and Grand Rapids, where several grasses are grown in turf in 10' by 10' plots side-by-side, the seaside bent has been observed to be severely attacked, while Colonial bent beside it did not have a spot of disease.

Metropolitan bent is resistant to snowmold. On the demonstration plots, where snowmold has appeared on other grasses, little has developed on this strain. At Wayzata, Minn., Metropolitan and an unnamed strain, found on the golf course in 1929, were growing side-by-side. The wild strain had 4 or 5 times as much disease as the Metropolitan strain.

Fescue is susceptible and on some golf courses where fescue greens were planted the grass has been eliminated by snowmold. This is one of the limiting factors in the growing of fescue greens in this country.

On a fairway near Grand Rapids several strains of creeping bent were growing together. Some of these were in large patches and several had been attacked by snowmold. One patch was observed that had a mixture of 2 strains of bent. One of the strains was severely diseased and the other was green. Figure 6, A shows this patch of grass.

#### DISCUSSION

Hiltner and Ihssen (6) found snowmold worse on strong vigorous plants and on manured soils or where straw had been applied. They recom-

mended grazing the fields in the fall because it reduced the amount of leaf area. Lindfors (8) also recommended reducing the amount of leaf area by later seeding, fall grazing, or cutting. Both investigators explain the increased severity of snowmold on the assumption that larger plants give off more heat because of the greater amount of respiration. However, the temperature under the snow does not vary from the freezing-point and is not higher where there is greater leaf area or where straw has been applied. The greater amount of snowmold under those conditions may be, in part, due to the ease with which the organism can spread from leaf to leaf. When the leaves are abundant and large, the mycelium overgrows the leaves and they become matted together to form a layer over the plants. Under this layer the organism can develop as long as temperature remains low, even after the snow has disappeared.

The large amount of organic matter in putting green turf enhances the disease, and where straw is applied and kept wet by the snow cover, there is an abundance of material on which the fungus can develop saprophytically. From this substratum the mycelium of the organism can spread to the leaves of the grass plants.

The absence of serious attacks of snowmold on winter grains in this country probably is due to the winter freezes that occur before snow falls. In Europe snow falls before many frosts have occurred and before the growth of the plants has stopped. In this country these freezes stop the growth of the plants and the tissue of the leaves becomes mature. When the plants are growing actively and the vegetation is soft and succulent, there is rapid assimilation and respiration. These processes are balanced and as the tissues mature they are slowed and there is a storage of carbohydrates and a loss of water from the cells. Such mature tissue is apparently not susceptible to attacks of the disease. But when the plants are still in an active vegetative state and snow occurs, the elimination of light stops assimilation; respiration continues, so that the carbohydrate reserves are reduced and the tissues may be a better medium for the growth of the fungus. Under these conditions the plants are more susceptible to attacks of the disease. On putting greens it has been apparent that strong growth of grass in the fall encourages attacks of snowmold. This is not due to increased temperature and cannot be explained in all cases by increase in leaf tissue because grass is clipped very short on greens in the fall. Plots treated with fertilizer were cut the same height as the nontreated plots, so that the amount of leaf area was almost the same. It is probable that the greater amount of disease is due to the immaturity of the tissue of the leaves, which makes it more favorable for the development of the fungus than the more mature tissue of plants that are not growing. On putting greens, grass stimulated by fertilizers late in the fall, is not allowed to mature before snowfall, thus making it susceptible to attacks of snowmold.

## SUMMARY

Snowmold occurs on winter cereals in northern Europe and on turf grasses in the United States and Canada. It is particularly important on golf courses in the United States and Canada. The disease occurs on turf in the form of irregularly circular patches of dead grass. These appear as the snow is melting and usually are from a few inches to a foot or more in diameter and may run together to cover large areas. The patches are whitish gray or may have a pinkish tinge. Individual plants have a bleached appearance and feel slimy when wet. On golf courses the disease occurs on Kentucky bluegrass, annual bluegrass, redbot, red fescue, creeping bent, velvet bent, and Colonial bent.

Snowmold on golf courses in this country was found to be caused by *Fusarium nivale* (Fr.) Ces. (*Calonectria graminicola* (Berk. and Brm.) Wr.). The organism grows well on oat-meal and potato agar on sterile grass clippings. Spores are formed on sporodochia when the culture is exposed to diffused light. Light changes the color of the mycelium from white to pink. Temperature studies showed that the organism grew at temperatures from 2° to 32° C. with an optimum of about 20° C.

Creeping bent, Kentucky bluegrass, redbot, red fescue, barley, rye, wheat, and oats were artificially inoculated in low-temperature moist chambers. The most rapid and injurious infections took place in chambers kept at 0 to 5° C. At 15 to 20° C. infections were slight and very slow.

The organism was found to enter the leaves through the stomata. Its progress through the tissue was intercellular until the cells began to collapse and then it became intracellular. After the mycelium became abundant in the tissue, sporodochia were formed on the leaf over the stomatal openings so that they occurred in rows on the leaf.

Records of temperatures under the snow showed that the organism grew at about the freezing-point. Observations indicated that climatic conditions greatly influenced the amount of snowmold. The conditions that favored attacks of the disease were: abundant moisture in the fall; snow falling on unfrozen ground; deep snow; and a prolonged cold, wet, spring.

Applying fertilizer late in the fall was found to make attacks of snowmold more serious. Where the soil had abundant organic matter or where the greens were covered with straw there was an increase of the disease.

Field experiments showed that the disease could be controlled by treating the turf in the autumn with corrosive sublimate or calomel at the rate of 3 oz. to 1,000 sq. ft. Applications of mercury fungicide at rates of 1 and 2 oz. to 1,000 sq. ft. were only partially effective.

It was found that there was a difference in the susceptibility to the dis-

ease in the grasses used on golf courses. Strains of grass growing side by side often have very different amounts of disease.

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# THE LONGEVITY OF THE LATENT AND VEINBANDING VIRUSES OF POTATO IN DRIED PLANT TISSUE<sup>1</sup>

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## INTRODUCTION

A review of the literature reveals only a few references that give definite statements regarding the longevity of the latent and veinbanding viruses of potato when dried in leaf or other plant tissues. It was shown by Johnson (5)<sup>3</sup> that the latent virus of potato was short-lived when separated from the living host. He says, "In most cases the survival is less than 20 days and frequently as low as 10 days. In general the virus survives longer in the drying leaf than in extracted plant juice."

As reported by Vanterpool (14), in his work with streak of tomato, "Portions of diseased plants were air-dried at room temperature until thoroughly dry. . . . In no single case did streak develop, but definite mosaic, usually of a very severe nature, invariably was produced when dried diseased material which had been stored for two, three, and nine months was used."

It was observed by Stover (12) that, "The potato element became non-infective in a short time after the death of the affected tissue, while the tomato element may remain infective for some time."

It was shown by Smith (11) that, "in filtered juice (tomato) Majestic mosaic remains infective for five and a half months, the longest period yet tested. Up-to-Date mosaic, however, was found to be inactive after 12 weeks, the filtered juice having been kept in dull light in paraffin-stoppered tubes."

In 1929, Johnson (6) again points out that the potato viruses are short-lived when placed *in vitro*. In working with several viroses of potato he concludes that ". . . The data indicate, however, that in the case of the crinkle mosaic virus a large part of the virus is inactivated at the end of six hours, and that it is all inactivated between 24 and 48 hours. In the case of rugose mosaic (extracted from potato foliage) and the leaf-rolling mosaic virus, inactivation is apparently not as rapid, but again little or no infection may be expected from the virus after aging from 24-48 hours. The mild mosaic virus appears to be the most sensitive. Aging for even

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<sup>3</sup> Reference made by number to "Literature Cited."

two hours *in vitro* appears to cause inactivation, although in one case a low percentage of infection was secured after aging for four hours. The mottled form of the virus from apparently healthy potatoes (transferred to tobacco) will resist inactivation for ten to twelve days. The rugose mosaic (spot-necrosis) virus from tobacco (transferred to tobacco) will also resist aging several days, consequently differing from its behavior when taken from potato."

In 1930 Samson (10) differentiated 2 viroses of Jimson weed. The milder type agrees with the latent ("mottle" of Johnson) from apparently healthy potatoes. "The more severe type . . . resembles the virus of Johnson's spot necrosis or rugose mosaic. Both of these Jimson weed viruses have survived 80 days aging *in vitro* in tomato juice at 10° C."

It was shown by Doolittle and Blood (2) that 3 distinct forms of tomato streak exist in Wisconsin. The form produced by the combination of tomato mosaic and the juice of the potato has a longevity *in vitro* of about 14 days. One of the other forms lives for at least 180 days.

As reported by Johnson (4) the "'Healthy potato' virus does not retain its infectivity in dried tissue (Table 2)." His experiments showed that dried virus-diseased tobacco, which had been aged for 9 months, 27 days, and 16 months, 7 days, respectively, gave negative results. He also points out that the mixed virosis of the latent ("Healthy potato") virus plus veinbanding was noninfective when dried for a similar period of time. He further showed that with the veinbanding virus "The infectivity is destroyed by drying at room temperature (Table 2)." The veinbanding virus was not recovered from extract of dried virus-diseased tobacco that had been aged for 9 months, 27 days and 17 months, 8 days, respectively.

Miss Jarrett (3) concludes that "glasshouse" streak differs from experimental streak (tobacco mosaic plus the latent virus) in that it responds to aging, *in vitro*, closely to Johnson's tobacco virus 1 and differs from potato mosaic in that the latter loses its power of infection after 6 months' storage *in vitro* in tomato or tobacco extract. She further states, "Extracts from plants which have been inoculated with two viruses, viz., experimental streak 1 and 2, have power to reproduce these diseases entirely for only 5 to 6 months, after which time it is assumed that the potato virus has lost its virulence, for the resulting symptoms are those of tobacco mosaic, or the mosaic of glasshouse streak only. After 12 months—no longer period has yet been tried—these symptoms appear regularly. . . . It is interesting to note that the virus of potato mosaic is less resistant than tobacco virus 1 or glasshouse streak to all three of these treatments."

In 1930 it was stated by Valteau and Johnson (13), "As the healthy potato virus does not appear to be able to withstand drying the possibility of a mixture with it '(true ringspot of tobacco)' seems to be eliminated."

As shown by Burnett and Jones (1), in a number of separate tests, the latent virus may be quite readily recovered from virus-infected potato, tomato, or tobacco leaf tissue after drying at room temperature for 10 to 46 days. Their records also show that the latent virus was recovered from virus-infected tomato and tobacco leaf tissue after being dried for periods of 77, 200, 233, and 286 days, respectively, and, moreover, that streak of tomato (latent plus tobacco mosaic) was recovered from dried tomato tissue that had been dried for periods up to and including 466 days. Likewise, the veinbanding virus was shown to remain active in dried plant tissue for at least 46 days.

In 1932 Johnson and Grant (8), working on the properties of different viruses, state, "With regard to the mottle form alone, the data for which are shown in parenthesis in table 7, there appears to be no evidence of any significant influence of the host species on the longevity *in vitro*." From the figures presented in that table it would appear that the spot necrosis viruses (veinbanding plus latent or rugose mosaic) are largely inactivated at the end of 20 days *in vitro*. The veinbanding virus (in the complex) is partly inactivated after two days with a more or less gradual diminution or a complete loss at the end of 20 days. A similar but decidedly slower inactivation is also noted for the latent virus.

#### MATERIALS AND METHODS

The experimental work was carried on in the greenhouse at the State College of Washington, Pullman, Washington, during the period from September, 1929, to March, 1933. Methods similar to those employed and reported in a previous article by Burnett and Jones (1) were followed throughout most of the experiments. A modified maceration method of inoculation as reported by Jones (9) was employed.

Juice from potato, tomato, and tobacco plants naturally infected or artificially inoculated with a single virus or combination of viruses was tested on tobacco or tomato plants to determine the presence of these viruses. The foliage from these infected plants was placed in open paper bags and allowed to dry in a section of the greenhouse under thermostatic control, the temperature generally ranging from 68°–75° F. Collections were made at various periods throughout the experiment and the dried inoculum was tested on tobacco or tomato plants at intervals of time in order to determine the longevity of the viruses in dried plant tissue. The John Baer variety of tomato and Connecticut Havana tobacco were used in these experiments. Because of the shortage of available space and the large number of series tested over a period of nearly 4 years, it was impossible to test all series at the same time. For this reason the various series were tested at varying intervals of time and then grouped and tabulated, as shown in the tables.

When a given inoculum failed to give positive symptoms on the test plants it was discarded and no further tests were made.

In some series only 2 plants were used as test plants. This was especially true when the inoculum had been dried but a short time. As the length of drying increased and the percentage of infection decreased in a given series, the test plants in the subsequent series were increased to 5, 10, or even 20. It is obvious that if the percentage of infection is low the recovery of the virus will be more likely if a greater number of test plants are used.

#### LONGEVITY OF THE LATENT VIRUS

Tests were made of the longevity of the latent virus of potato when dried in potato, tomato, or tobacco leaf tissue. The determinations were made by using the macerated leaf tissue after various periods of drying to make inoculations into young tobacco and tomato plants. The results are presented in table 1, with an interpretation of the figures offered in a footnote.

Using the fresh leaf tissue, a number of different sources were tested for each host: 62 series for potato; 27 for tomato; and 42 for tobacco. In the potato series 57 different lots inoculated on 229 tobacco plants gave 197 with symptoms, while 5 series tested on 2 plants each gave negative symptoms. Of the tomato series 20 were positive and 7 negative, and for tobacco 38 were positive and 4 negative. For leaf tissue dried 3 days or for longer periods, in general, a lesser number of sources or lots were used in a similar way for each of the hosts. If there was any doubt whatever regarding the symptoms on any series the results were recorded as negative. This may account for the negative results when fresh inoculum was used.

A further analysis of tables 1 and 2 also reveals the fact that, with the fresh inoculum, the percentage of series giving positive results is 72.2 to 91.9 per cent, whereas the percentage of test plants showing positive symptoms, in these series, varies from 82.4 to 86.4 per cent. In general, the longer the inoculum was dried the lower was the percentage of infection. This also accounts for the increase in the number of series giving negative results as the length of time of drying is increased. There is insufficient evidence from the data presented to state that the latent virus will retain its infectivity longer in one host than in another. At the end of 3 days the percentage of infection varied from 95 to 100 per cent.

When potato foliage that had been dried for 3 to 28 days was tested, as shown in table 2, 66.6 per cent of the series gave positive results. For the periods 29-50, 132 and 263 days, the recovery was 50, 1.7, and 100 per cent, respectively. These figures are not so erratic as they may seem when it is noted in table 1 that only one series of the 57 tested gave positive re-

TABLE 1.—*Results of the longevity tests of the latent virus of potato when dried in potato, tomato, or tobacco leaf tissue and reinoculated on tobacco and tomato plants*

No. of days dried	Recovery of the virus from dried foliage of					
	Potato		Tomato		Tobacco	
	Positive	Negative	Positive	Negative	Positive	Negative
None "Fresh inoculum"	$\frac{57-229^1}{197}$	$\frac{5-10}{0}$	$\frac{20-140}{121}$	$\frac{7-35}{0}$	$\frac{38-154}{127}$	$\frac{4-20}{0}$
3	$\frac{8-40}{40}$	$\frac{4-20}{0}$	$\frac{12-60}{57}$		$\frac{12-60}{60}$	
10	$\frac{8-40}{28}$	$\frac{4-20}{0}$	$\frac{10-50}{31}$	$\frac{2-10}{0}$	$\frac{11-55}{43}$	$\frac{2-10}{0}$
11 to 13	$\frac{4-20}{6}$	$\frac{2-10}{0}$	$\frac{4-20}{9}$	$\frac{2-10}{0}$	$\frac{19-175}{91}$	$\frac{5-55}{0}$
17	$\frac{8-40}{22}$	$\frac{4-20}{0}$	$\frac{10-50}{35}$	$\frac{2-10}{0}$	$\frac{10-50}{31}$	$\frac{4-20}{0}$
20					$\frac{10-100}{29}$	$\frac{11-110}{0}$
28	$\frac{4-20}{17}$	$\frac{2-10}{0}$	$\frac{4-20}{5}$	$\frac{2-10}{0}$	$\frac{3-15}{4}$	$\frac{3-15}{0}$
29 to 50	$\frac{8-40}{18}$	$\frac{8-40}{0}$	$\frac{11-55}{24}$	$\frac{7-45}{0}$	$\frac{10-50}{31}$	$\frac{6-30}{0}$
72 to 175	$\frac{1-10^2}{1}$	$\frac{56-560}{0}$			$\frac{2-20^3}{4}$	$\frac{13-130}{0}$
250 to 325	$\frac{1-10^4}{2}$			$\frac{2-40}{0}$	$\frac{1-20^5}{6}$	$\frac{10-140}{0}$
326 to 775		$\frac{13-170}{0}$		$\frac{35-540}{0}$		$\frac{9-110}{0}$

<sup>1</sup> First number of numerator represents total number of series or sources of inoculum; second number, the total number of plants inoculated; and the denominator represents the number of plants showing symptoms of the latent virus.

<sup>2</sup> Dried 132 days.

<sup>3</sup> Dried 72 days.

<sup>4</sup> Dried 263 days.

<sup>5</sup> Reported by Burnett and Jones (1)—dried 286 days.

sults for the period of 72 to 175 days, with a 10 per cent infection, and only one series tested gave positive results for the 263 day period with a 20 per cent infection. The percentage of plants infected, when inoculated with the potato foliage, varied from 100 for the 3 day period to 10 for the

263 day period, the latter being the longest period for which the latent virus was recovered from dried potato foliage. With a few exceptions there is a rather gradual reduction in the percentage of infection as the length of drying is increased.

In a similar manner, the tests with tomato foliage, as shown in tables 1 and 2, gave some variations in the percentage of series giving positive results and in the percentage of plants showing symptoms. Only 72.2 per cent of these series with the fresh inoculum showed recovery; 100 for the 3-day test and a fairly gradual diminution to 61.1 for the 29-50-day test.

TABLE 2.—*Positive recovery of the latent virus from dried material in per cents of positive series and positive plants. Based on results in table 1.*

No. of days dried	POTATO <sup>1</sup>		TOMATO		TOBACCO	
	Per cent series positive	Per cent plants positive	Per cent series positive	Per cent plants positive	Per cent series positive	Per cent plants positive
None "Fresh inoculum"						
3	91.9	86	72.2	86.4	90.4	82.4
10	66.6	100	100	95	100	100
11 to 13	66.6	70	83.3	62	84.5	78.1
17	66.6	30	66.6	45	79.1	52
20	66.6	55	83.3	70	71.4	60.2
28	.....				47.5	29
29 to 50	66.6	85	66.6	25	50	26.6
72 to 175	50	45	61.1	43.6	62.5	60.2
250 to 325	1.7 <sup>2</sup>	10		...	13.3	20
326 to 775	100 <sup>3</sup>	20	0		9.09	30
	0		0		0	

<sup>1</sup> The first column under each host represents the per cent of series giving positive results; the second column under each host represents the per cent of inoculated tobacco plants in the positive series showing symptoms of the latent virus.

<sup>2</sup> Dried 132 days.

<sup>3</sup> Dried 263 days.

There was some fluctuation in the number of plants showing positive symptoms, with a greater percentage of infection with the fresh inoculum and the material dried for 3 days; but, again, it is evident that the foliage that had been dried longer generally gave a lower percentage of recovery. The virus was not recovered from tomato foliage that had been dried more than 50 days.

When tobacco foliage was tested, as shown in tables 1 and 2, the fresh inoculum showed 90.4 per cent of the series positive, 100 per cent for the

3-day test, with a more definite and gradual decline to 9.09 per cent for the 286-day test. In the latter case when tobacco foliage had been dried 286 days, 6 plants of the 20 inoculated gave positive symptoms of the latent virus.

After the inoculum had been dried for 72 days or more, only 5 separate series, 2 with the potato foliage and 3 with tobacco foliage, gave positive results of recovery of the virus, and the infection varied from 10 to 30 per cent.

There was no consistent or significant difference in the longevity of the latent virus when secured from apparently healthy potatoes or from potatoes that showed definite symptoms of rugose mosaic, crinkle mosaic, leaf roll or other viroses. Definite data are not presented in the tables, but naturally infected rugose-mosaic potato plants (latent plus veinbanding), when tested on tobacco plants, reveal the fact that, in general, the latent virus withstands aging longer than does the veinbanding virus. Similar results were presented by Johnson and Grant (8) for the spot-necrosis viruses (rugose mosaic) when tested *in vitro*.

Whether virus-infected plant parts are dried rapidly or slowly may determine to a large extent how long a specific virus may be recovered from such infected host tissue. Definite figures are not presented, but in working with numerous collections, it appeared that the virus retained its virulence much longer in infected plant tissue when dried rapidly than when dried slowly or subjected to fluctuating air humidity or to alternate wetting and drying in the soil.

#### LONGEVITY OF THE VEINBANDING VIRUS

A corresponding series, similar to that presented for the latent virus, was conducted to determine the longevity of the veinbanding virus. Experimental results that show the recovery of the veinbanding virus from dried foliage of potato, tomato, and tobacco are presented in table 3. The tabulations are arranged according to the same plan as in table 1. The infection with fresh inoculum approaches nearly 100 per cent. There was little additional inactivation at the end of 3 days. At the end of 10 days there was a noticeable decrease in the percentage of recovery. This was likewise true for the 17-day test. The veinbanding virus was not recovered from tomato foliage that had been dried more than 17 days. It was recovered from dried potato and tobacco foliage at the end of 31 and 50 days, respectively, with approximately 70 per cent of the test plants showing positive symptoms for the 50-day period, but it was not recovered when the foliage had been dried for 100 days or more.

The source of the veinbanding virus did not appreciably influence its longevity in the dried foliage of the 3 hosts. The percentage of recovery

TABLE 3.—*Results of the longevity tests of the veinbanding virus when dried in potato, tomato, or tobacco leaf tissue and reinoculated on tobacco plants*

No. of days dried	Recovery of the virus from dried foliage of					
	Potato		Tomato		Tobacco	
	Positive	Negative	Positive	Negative	Positive	Negative
“Fresh inoculum”	$\frac{19-38^1}{34}$	$\frac{2-3}{0}$	$\frac{2-4}{4}$	...	$\frac{48-85}{81}$	$\frac{2-10}{0}$
1 to 2	..	..	..	..	$\frac{2-10}{9}$	..
3	$\frac{6-30}{29}$	$\frac{2-10}{0}$	$\frac{5-25}{19}$	$\frac{3-15}{0}$	$\frac{9-45}{44}$	..
4 to 8	..	..	..	..	$\frac{5-25}{15}$	..
9	$\frac{2-10}{5}$	$\frac{2-10}{0}$	$\frac{1-5}{5}$	$\frac{3-15}{0}$	$\frac{4-20}{10}$	..
10	$\frac{6-30}{22}$	$\frac{3-15}{0}$	$\frac{2-10}{7}$	$\frac{7-35}{0}$	$\frac{6-30}{18}$	$\frac{4-25}{0}$
11 to 13	..	..	..	..	$\frac{3-15}{7}$	$\frac{4-40}{0}$
14 to 16	..	..	..	..	..	$\frac{4-40}{0}$
17	$\frac{5-25}{18}$	$\frac{3-15}{0}$	$\frac{1-5}{1}$	$\frac{8-40}{0}$	$\frac{4-20}{13}$	$\frac{6-30}{0}$
18 to 24	..	..	..	..	..	$\frac{17-175}{0}$
31	$\frac{3-15}{5}$	$\frac{6-30}{0}$	..	$\frac{8-40}{0}$	$\frac{1-5}{2}$	$\frac{7-35}{0}$
50	$\frac{4-40}{28}$	$\frac{2-10}{0}$	..	$\frac{2-20}{0}$	$\frac{2-20}{15}$	$\frac{2-20}{0}$
100 to 362	..	$\frac{56-730}{0}$	..	$\frac{22-320}{0}$	..	$\frac{14-160}{0}$

<sup>1</sup> First number of numerator represents total number of series or sources of inoculum; second number, the total number of plants inoculated; and the denominator represents the number of plants showing positive symptoms of the veinbanding virus.



from the tomato foliage appears to be somewhat less than from the potato or tobacco foliage. The possibility of recovery from tomato appears to be somewhat lower, but this fact is not necessarily significant when the number of tests presented are taken into consideration. With a great number of tests this fact may or may not be borne out.

LONGEVITY OF THE LATENT VIRUS WHEN IN COMBINATION WITH  
TOBACCO MOSAIC (STREAK OF TOMATO)

Results of the longevity tests for the latent virus in combination with tobacco mosaic (streak of tomato), when dried at room temperature in potato, tomato, or tobacco leaf tissue and reinoculated in tomato plants are presented in table 4. Tests up to and including 466 days, as reported by Burnett and Jones (1), are not included in this report.

The latent virus used in these tests was originally secured from potato plants naturally infected with one of the following diseases; crinkle mosaic, latent (apparently healthy potatoes), leaf roll, rugose mosaic, and spindle tuber. Potato seedlings as well as tomato and tobacco plants were inoculated with tobacco mosaic and the latent virus from one of the above sources. After the viruses had been given time to become systemic in the plants or had given definite symptoms, the foliage was removed and dried as reported under "Materials and Methods." This foliage, after being dried for variable periods of time, was retested on tomato plants as indicated in table 4. The tabulated results are not intended to show the percentage of tobacco mosaic recovered but to demonstrate the recovery of the latent virus. In nearly all of the tests, however, the recovery of tobacco mosaic from tomato and tobacco foliage was 100 per cent. When the inoculated potato seedling foliage was tested the percentage of recovery of tobacco mosaic was very low. The entire foliage of the inoculated potato seedlings was collected and used as a source of inoculum. Since the potato is often resistant to systemic infection with tobacco mosaic, it is possible that when tobacco mosaic was recovered in the test plants it was obtained from the point of local infection of the inoculated leaf.

The tabulations are arranged according to the same plan as in table 1. It will be noted that 2 plants of the 20 inoculated, for the period of 325 to 425 days, with inoculum from the potato foliage gave positive results. This is the longest time that the latent virus was found to remain infective when dried in potato foliage. In a similar manner when the combination of the latent virus and tobacco mosaic was dried in tobacco foliage it retained the infective agent of the latent virus for 618 days. This gave in 2 separate series 20 per cent infection (the production of streak on tomato), which is represented by 8 out of 40 plants showing positive symptoms. When foliage from tomato plants, which exhibited true streak (latent plus tobacco mosaic), was

room temperature in potato, tomato, or tobacco leaf tissue and reinoculated on tomato plants

No. of days dried <sup>1</sup>	Dried in host	Recovery of the latent virus when the following virous diseases of potato were used as the original sources of inoculum									
		Crinkle mosaic		Latent		Leaf roll		Rugose		Spindle tuber	
		Pos. <sup>2</sup>	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
325 to 425	Potato seedling		1-10 <sup>3</sup> 0		2-20 0	1-20 <sup>4</sup> 2			9-130 0		
	Tomato	4-7 <sup>0</sup> 7	13-21 <sup>0</sup> 0	2-4 <sup>0</sup> 8	6-7 <sup>0</sup> 0	1-20 1	2-30 0	4-80 12	14-25 <sup>0</sup> 0	1-20 1	4-70 0
	Tobacco		1-1 <sup>0</sup> 0			1-20 2			3-5 <sup>0</sup> 0		
550 to 750	Potato seedling				1-10 0		1-20 0		1-20 0		
	Tomato	3-6 <sup>0</sup> 10	2-4 <sup>0</sup> 0			1-20 <sup>5</sup> 1	1-20 0		1-20 0		
	Tobacco		1-20 0	2-40 <sup>5</sup> 8					1-20 0		
758	Tomato	1-20 <sup>6</sup> 3	2-4 <sup>0</sup> 0								
826	Tomato	1-20 <sup>6</sup> 2									
1202	Tomato	1-20 <sup>6</sup> 3									
1251	Tomato	1-20 <sup>6</sup> 2									

<sup>1</sup> Longevity tests of the latent virus (streak of tomato) up to and including 466 days previously reported by Burnett and Jones (1).

<sup>2</sup> Pos. = symptoms evident; Neg. = no symptoms.

<sup>3</sup> First number of numerator represents the total number of series or sources of inoculum; second number the total number of plants inoculated; and the denominator represents the number of plants affected with streak. Nearly 100 per cent of plants gave positive symptoms of tobacco mosaic, except inoculum from potato foliage which in general gave negative results.

<sup>4</sup> Dried 352 days. <sup>5</sup> Dried 618 days. <sup>6</sup> Inoculum from the same source.

dried and retested on tomato, the latent virus was sufficiently virulent to produce 10 to 15 per cent infection after being dried for 1251 days, or approximately  $3\frac{1}{2}$  years. This particular streak inoculum was collected on July 30, 1929, by Dr. L. K. Jones from tomato plants that had previously been inoculated with the latent virus from potatoes showing crinkle mosaic, and with tobacco mosaic. This inoculum was dried very rapidly when collected because of the prevalent low humidity and the high temperature. At that time it was not uncommon to have the temperature reach  $95^{\circ}$  to  $100^{\circ}$  F. during the day-time. This inoculum was still infective when the last test was made on January 2, 1933.

It has been noted throughout these experiments that when the inoculum is dried more rapidly there is a tendency for the latent virus to retain its virulence for a longer time. A similar result was obtained with tobacco mosaic by Johnson and Ogden (7) who state that, "Moist and well aerated soils favor the inactivation of the virus as compared with dry, compact or waterlogged soils." With fresh streak inoculum it is not uncommon to obtain 100 per cent infection of streak on tomato plants. As the period of time of drying is increased the percentage of recovery is reduced. With inoculum that had been dried for 6 months or more, where the recovery of the virus was secured, the results showed from 5 to 20 per cent infection. If only a small number of plants are inoculated, there is much less probability of recovering the latent virus if the percentage of infection is low.

It is interesting to note (Tables 1 and 4) that when the latent virus was dried alone in plant tissue it did not survive so long as when dried in the presence of tobacco mosaic (streak). By referring to table 1 it will be noted that the longest period for which the latent virus was recovered from dry plant tissue when used alone was 286 days. When the latent virus (as secured from potatoes with crinkle mosaic) was combined with tobacco mosaic in tomatoes to produce streak it was found infective after being dried for a period of 1251 days, or approximately  $3\frac{1}{2}$  years (Table 4). Other data from table 4 also show that the latent virus when combined with tobacco mosaic retained its infectivity longer than 286 days, as pointed out for the latent virus when dried alone. It is not clear just what influence the tobacco mosaic has on the longevity of the latent virus when the 2 viruses are dried in combination in plant tissue. From the results as indicated in these experiments, it would appear that the latent virus remains virulent much longer when found in combination with tobacco mosaic than when dried alone in leaf tissue. The 2 tomato plants that gave positive streak symptoms after having been inoculated with the streak inoculum that had been dried for a period of 1251 days were further tested on *Datura stramonium* to determine definitely the presence of the latent virus. The 10 *Datura* plants, thus inoculated, gave the local lesions on the inoculated leaves characteristic of tobacco

mosaic, while on the younger foliage only the mottle characteristic of the latent virus appeared. This gave further evidence that the latent virus was present and infective in the streak inoculum that had been dried for 1251 days.

A further test was made that demonstrates the presence of the latent virus in the above streak material. Five healthy potato plants of the Early Rose variety, which had been tested and found to be free of the latent as well as other viruses, were inoculated with the streak inoculum. Following a 2-week period of incubation, the foliage from each of the 5 potato plants was tested on tomato plants to which fresh tobacco mosaic was added. All of the tomato plants thus inoculated gave a severe type of streak.

#### SUMMARY

The latent virus when dried alone in plant tissue was found infective after being dried for 286 days in tobacco, 263 days in potato, but for not over 50 days in tomato. These results were obtained from inoculations involving 510 series of dried inoculum on 3743 tobacco and tomato plants.

The veinbanding virus, when dried alone in plant tissue, was found infective after being dried for 50 days in potato and tobacco but for only 17 days in tomato. These results were obtained from inoculations in which 329 series of dried inoculum on 2355 tobacco plants were used.

The latent virus, when dried in foliage in combination with tobacco mosaic (streak of tomato), remained infective longer than when dried alone. It (in the combination) was recovered from potato, tomato, and tobacco foliage that had been dried for periods of 352, 1251, and 618 days, respectively. These results were obtained from inoculations using 86 series of dried inoculum on 1510 tomato plants.

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# FREQUENT ASSOCIATION OF PHYTOMONAS MELOPHTHORA, WITH VARIOUS STAGES IN THE LIFE CYCLE OF THE APPLE MAGGOT, RHAGOLETIS POMONELLA<sup>1</sup>

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## INTRODUCTION

*Phytomonas melophthora* A. and R., which causes rot of ripe apples, has been studied in relation to various stages in the life cycle of the apple maggot, *Rhagoletis pomonella* Walsh. This work was undertaken because of the various indications (reviewed by Allen and Riker (2)), of a frequent relation between the larvae of this insect and the rot organism. It seemed desirable to determine, if possible, how the larvae and the bacteria became associated and the frequency of this association throughout the life cycle of the insect.

The economic importance of the combination of *Phytomonas melophthora* with the apple maggot is manifest both in the orchard and in storage. Under natural conditions in the orchard the dissemination of the bacteria and the mode of their entry into the fruit are apparently dependent upon the insect. Under storage conditions subsequent development of rot in the fruit seems associated with the presence of maggot infestation. Consequently, further study of this relation appeared desirable.

The problem has been studied primarily by means of certain bacteriological methods applied to various stages of the insects and to their immediate environment. This type of study has already shown its value in the excellent work by Leach (6) and Johnson (4) on the relation of certain insects to the spread of soft-rot bacteria. The technic employed by the writers is reviewed briefly.

## MATERIALS AND METHODS

The materials used in these studies included (1) the various available stages in the life cycle of the insect and (2) the accompanying decay in apple tissue, as listed in table 1. Infested fruits were obtained in 1931 and 1932 from Gays Mills, Madison, Sparta, and Winneconne, Wisconsin. Adult flies and the fruits that contained both eggs and the ovipositor punctures were collected at Gays Mills. Several varieties of maggot-infested apples were used in the studies: viz., Dudley, McMahon, Snow, Wealthy and Yellow Transparent.

<sup>1</sup> Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies have been made in cooperation between the Departments of Economic Entomology and Plant Pathology.

Isolations from apple tissue were made in accordance with the usual laboratory technique as employed by Allen and Riker (2), except as noted.

Isolation studies from the various stages of the insect involved variations in procedure. Adult flies, eggs, egg-shells, and larvae were placed in broth and removed after 20 minutes. The broth was incubated 48 hours at room temperature before dilution plates were poured. Suspensions from the broth were transferred into healthy apples. When bacterial decay developed in the apple tissue, poured-plate isolations were made on nutrient glucose agar. Flies were killed with chloroform or ether before crushing in tubes of nutrient glucose broth.

Methods for treatment of flies, eggs, and larvae with surface disinfectants were as follows: The material was placed in 70 per cent alcohol for 3 minutes to remove air, washed in sterile water and transferred to bi-chloride of mercury, 1 to 1000. After 5 minutes it was washed in sterile water and placed in a tube of broth for 20 minutes. Then it was transferred to another tube of broth and crushed. The bacteria on the surface, reached by this method, were certainly killed, but the possibility remains that those embedded in slowly soluble material upon the surface may have escaped injury. The natural openings make it difficult to differentiate between surface and partial internal sterilization. However, in the data that are presented later, the interpretation is made that, when the tubes of broth in which the material remained for 20 minutes remained sterile, any bacteria subsequently recovered came from inside. The development of growth in a check broth tube was interpreted to mean that microorganisms occurred on the surface of the material and no further isolations were attempted. When growth appeared in the tube where the insect was crushed, inoculations with the broth were made into apples and reisolations were made as previously described (2).

Eggs, which were nontreated and incubated to obtain developing larvae, were removed from apple tissue and transferred aseptically to dark moistened blotting paper within small sterilized vials. These vials were kept slightly above room temperature. As larvae hatched, they were transferred aseptically to needle pricks in surface-sterilized fruits. As decay developed in the apples, isolation studies were made of the rot that developed adjacent to the larval burrows. Certain difficulties of technique remain to be overcome with eggs treated with a surface disinfectant, for only 2 out of 25 trials were successful. In these 2 successful attempts, the larvae from treated eggs died a couple of days after being placed in apple tissue. No rot developed in the surrounding fruit tissue.

Several methods were tried before successful isolation of the organism was obtained from the pupal stage. The following method gave the most satisfactory result. Puparia were removed from soil and placed in 5 cc.

of physiological salt solution for 20 minutes with frequent agitation. This salt solution was diluted approximately 1 to 6, 1 to 35, and 1 to 200. One cc. from each dilution was placed into each of 3 Petri dishes with glucose yeast-infusion mineral-salt agar. This medium was similar to that employed by Wright (7). Its composition was glucose, 10 grams; magnesium sulphate ( $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ ), 0.2 grams; sodium chloride ( $\text{NaCl}$ ), 0.2 grams; dipotassium phosphate ( $\text{K}_2\text{HPO}_4 \cdot 3 \text{H}_2\text{O}$ ), 0.1 gram; calcium chloride ( $\text{CaCl}_2$ ), 0.1 gram; 10 per cent yeast infusion, 100 cc. and distilled water 900 cc. The reaction was adjusted to pH 6.8.

Puparia were also treated in disinfectant as described earlier, except that they were previously washed 3 times in tubes of sterile salt solution. Instead of 3 dilutions as used earlier, 8 successive dilutions were made to approximately 1 to 1,000,000. Three plates were poured from each dilution, making a total of 24 plates from each puparium. This relatively large amount of work was performed because it increased the chances for success.

The pathogenicity of the cultures isolated was determined in each case on one of several varieties that included Delicious, McIntosh, Wagner, and Yellow Transparent.

The results secured with these methods are given briefly in the following pages.

#### RESULTS

*Phytophthora melophthora*, the cause of bacterial storage rot of apples in Wisconsin, has been found associated with all stages examined in the life cycle of the insect, *Rhagoletis pomonella*. The life cycle of this insect, with its accompanying plant pathogen, has been followed in material from the orchards at Gays Mills. Certain parts of the cycle were studied with material from other sections of the State. The results of isolation studies from the various stages of the insect and from corresponding decay in apples are reported in detail in the following pages and are summarized in table 1. The same sequence of the work on different forms of the insect and associated apple tissue is followed both in the text and in this table.

#### ADULT FLIES

The adult flies used in these experiments were commonly found harboring *Phytophthora melophthora* in such quantities that they were readily isolated by methods outlined above. The reasons for suspecting the adult fly as a vector in the production of this disease were (1) the rot that sometimes followed the wound produced during oviposition in relatively ripe fruit (Fig. 1, C), and (2) the association of the apple maggot with rot, as considered earlier (2).

The flies examined were secured, 13 from the orchard and 12 from a series of emergence cages previously placed over piles of infested fruit.



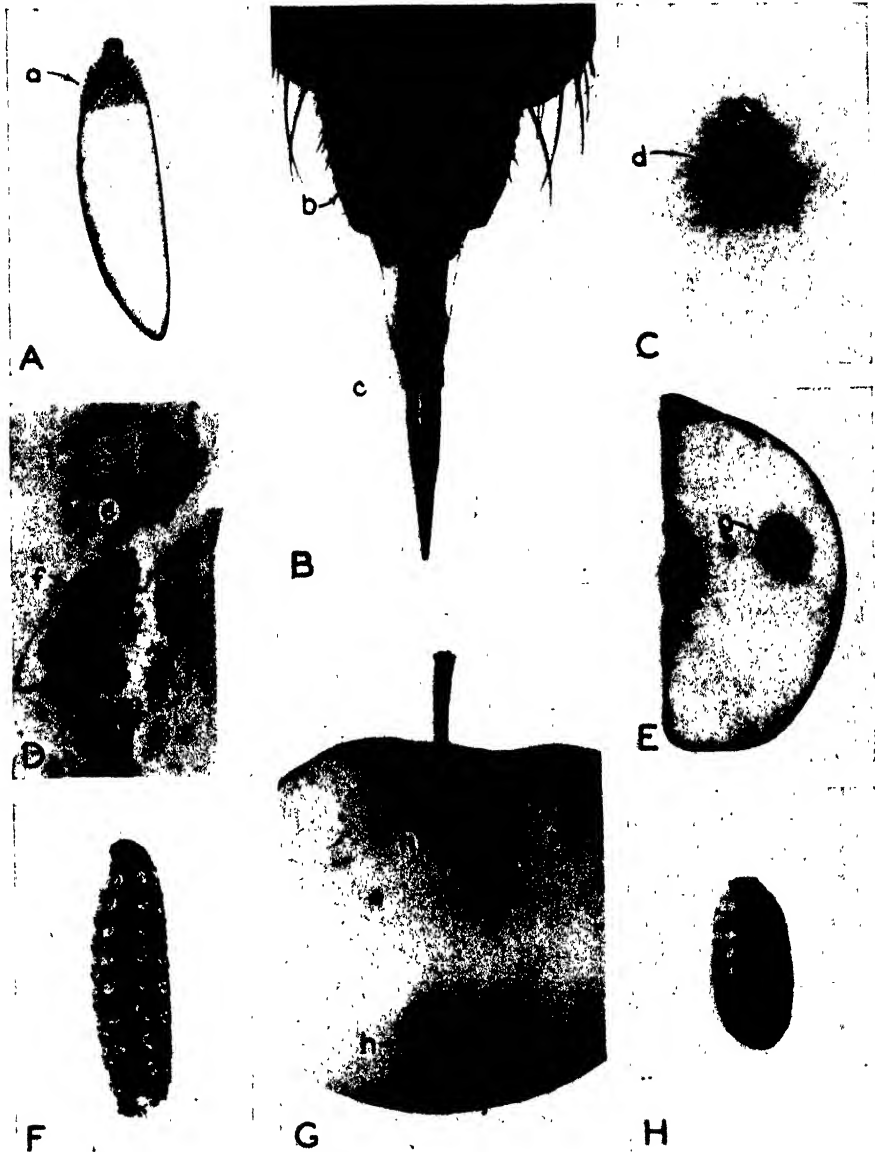


FIG. 1. A. Egg of the apple maggot and reticulation, *a*, at posterior end.  $\times 51$ . B. The posterior of a female fly, showing the end of the abdomen, *b*, and the partially extended ovipositor, *c*.  $\times 23$ . C. Egg puncture viewed from the surface of a ripe apple. Decayed tissue, *d*, appears about the puncture.  $\times 15$ . D. The egg, *f*, at normal position in apple tissue, showing ovipositor or egg puncture, *e*.  $\times 31$ . E. Rot, *g*, about larval burrow in apple.  $\times 1\frac{1}{2}$ . F. Mature larva.  $\times 6$ . G. Rot, *h*, about larval exit holes in apple.  $\times 1\frac{1}{2}$ . H. Pupa.  $\times 4$ .

Two broods of flies, some of which were obtained from 2-year-old puparia, were involved in the collection as explained by Allen and Fluke (1). The age of the flies collected in the orchard was uncertain, but only 1-day-old flies were used from the emergence cages.

Isolations were made to determine whether the flies were actually carrying the bacteria. The flies were subjected to the fumes of either chloroform or ether before the isolations. Both male and female flies were used for dilution plates either with or without surface treatment with a disinfectant as follows: Eight of 10 trials with nontreated female flies were positive for *Phytomonas melophthora*. Three trials with treated females were all positive. Four of 6 trials with nontreated male flies were positive. All of 6 trials with treated males were positive. These results are summarized in table 1. The larger percentage of positive results from the treated flies was perhaps the result of freedom from troublesome contaminations. Since about half of the males and females were only a day old and were from emergence cages it is suggested that they carried the bacteria either from the puparia or perhaps from the surrounding material. This question is considered further in the next paragraph and in connection with studies of the puparia. From these studies it appears that *Phytomonas melophthora* may be carried both internally and externally by the mature fly in quantities sufficiently large to account for the observed dissemination of the bacteria in Wisconsin orchards. This raises the question concerning how the association occurred.

Soil isolations were attempted in an effort to learn whether the flies became contaminated as they emerged. Four soil samples obtained within emergence cages were examined. Each soil sample was collected from the surface 2 inches in a sterile glass vial, mixed and divided into approximately 2 equal portions. One portion was plated in nutrient dextrose agar in triplicate from 6 dilutions that ranged up to 1 in 2,000,000. Part of the other portion of each sample was introduced into apple according to the method of Ark (3) with the exception that ripe Yellow Transparent apples were substituted for green pears. The negative results secured, perhaps because of inadequate technique, are not interpreted to mean that the soil does not harbor *Phytomonas melophthora*, but that, under the conditions of the above experiment, this organism was probably not present in the soil in relatively large quantities. Since more than three-fourths of the flies emerging from this soil carried the pathogenic bacterium, it appears more probable that the insects carried the bacteria from the puparia. How the flies transmitted the bacteria to the eggs was next considered.

#### Ovipositor Punctures

The ovipositor puncture was studied to determine the frequency of its inoculation. Five different varieties of apples were collected from 4

sources in the State, as mentioned earlier. The wounds made during oviposition, (Fig. 1, C) were located with binoculars. With sterile instruments the tissue of the fruit was cut away exposing the ovipositor puncture, *e*, and egg, *f* (Fig. 1, D). Isolations were made from the wound according to methods previously discussed. Results of isolations from 22 ovipositor punctures revealed that 10 of these contained *Phytomonas melophthora*. In connection with these results one should recall that relatively green apple tissue is not a favorable medium for the rot bacteria (2). These results suggest that bacteria may be transferred by means of the ovipositor (Fig. 1, B) and that, when conditions are favorable, rot may develop in the fruit following injuries made by the fly. Only a small percentage of the punctures were found devoid of eggs or egg-shells. The rate at which rot developed about the wound appeared to be influenced by the maturity of the fruit.

The inoculation of the ovipositor puncture might be accounted for by the habit of the adult fly. The ovipositor is extended and forced through the epidermis by several up and down movements. Any bacteria present on the ovipositor may therefore be smeared over the surrounding apple tissue. Following egg deposition, the ovipositor is generally cleaned by the rear legs of the insect before it is withdrawn into the abdomen. This cleaning habit also is performed in the same manner following defecation and can perhaps explain the distribution of rot bacteria over the ovipositor. Knight (5) has suggested that 2 pathogenic fungi were carried on the ovipositor of the apple maggot.

### Eggs

Eggs of the insect (Fig. 1, A and D, *f*) were deposited a short distance under the epidermis of the apple fruit. Fifteen eggs were dissected from the apple parenchyma and isolations made from surface washings, according to the above-described technic. The results of these isolations showed that eight of the eggs were contaminated with *Phytomonas melophthora*. The projections on the proximal end of the egg (Fig. 1, A, *a*) apparently serve both to prevent any reverse movement of the egg and perhaps to harbor bacteria.

Eggs from captive flies were obtained by placing pairs of flies in a glass rearing cage together with a ripe apple, and a little yeast-water honey mixture. After the adult had been observed to deposit the egg, the fruit was removed from the cage, the egg dissected from the host tissue and, examined as earlier described. Three eggs were successfully extracted and isolations made from their surfaces without injury to the egg. In each attempt *Phytomonas melophthora* was recovered. In addition, 2 others were successfully obtained and treated with disinfectants without killing the contained embryos. The larvae were subsequently hatched and were found

to be free from *Phytomonas melophthora* as described later. While the number is too small for conclusions, this evidence suggests that the bacteria do not occur inside the egg. The number of eggs employed is small because of the difficulty already mentioned that was experienced in removing them from the apple tissue and in handling them without injury.

#### Egg Shells

Egg shells found in apple tissue were treated in the same manner as the eggs. Larvae had emerged from the eggs after 4 to 6 days. Results of isolations from 24 egg shells showed that one-third of them carried the rot bacteria. It appears from the evidence available that as the larva emerges from its shell, it may frequently make contact with the apple-rot bacteria.

#### Larvae from Orchard

Young larvae were collected in apples taken from the orchard. Isolations made from 13 such larvae, not treated with surface disinfectants, showed that 3 of them were carrying the bacteria. It is probable that when the bacteria were present they were unable to increase in number much, if any, because of the relatively unfavorable condition in the green fruit. Similar larvae treated with surface disinfectants gave the same results from the same number of trials as those not treated. This indicated that the bacteria were present inside as well as on the surface of the larvae. Since the early life of the larvae is usually spent in relatively green fruit, decay rarely develops about the wounds made during oviposition or about the egg. The larvae hatch, but development is retarded until the fruit reaches a relatively mature condition. The number of bacteria available for isolation from such larvae as have hatched within 2 or 3 days, therefore, is quite small. If the parasitized fruit is removed from the tree while still in a "green" condition, further progress of both the larvae and rot seems to be dependent on the physiological processes of ripening and can be hastened or retarded by manipulation of the storage conditions. Larvae developed rapidly in ripe stored fruit usually amid conspicuous infection of the host parenchyma (Fig. 1, E, g). The above observations correspond to those following artificial inoculations on fruits in various stages of maturity (2).

Mature larvae (Fig. 1, F) from fallen fruit in the orchard were examined. Isolations from 13 of this group revealed that all contained *Phytomonas melophthora*. Thirteen similar larvae, which were treated with disinfectants, also revealed that all carried the plant pathogen. These trials showed that the mature larvae of this insect carried the rot-producing pathogen in relatively large numbers. While it was noted that the larvae apparently preferred the decayed portion of the fruit, no investigations

were undertaken on the relation of the products of decay to the nutrition of the insect.

Larvae from captive flies were obtained from the eggs laid in insect cages. Nontreated larvae from this source were allowed to develop normally in the apple tissue until they neared maturity. Six of them were then crushed in broth. In each trial *Phytomonas melophthora* was recovered from the larvae in large quantities. Larvae from nontreated eggs of captive flies also were hatched on sterile blotting paper. The larvae were placed in broth for 20 minutes and then transferred to sterile apple tissue. Rot was produced in each of these trials and the bacteria were recovered from both the apple tissue and the tubes of broth. These larvae pupated in the usual manner. Larvae from treated eggs of captive flies were hatched as above and placed in broth for 20 minutes and then into healthy apple tissue. No cloudiness appeared in the broth after 2 weeks' incubation and the larvae were assumed to be free from bacteria. After 3 days both of the insects used in this experiment had burrowed in the apple tissue for a few centimeters and had died. There was no apparent increase in size of the larvae. No signs of decay were detected in the apple tissue.

The rot that developed about the larvae from captive flies also showed the presence of the typical rot bacteria in each of 3 trials. No difference was observed in the character of the rot observed in the insect cages from that found in the orchard.

#### Larval Burrows

Larval burrows (Fig. 1, E, g) in a variety of stages of decay and development were tested for the presence of *Phytomonas melophthora* during the course of these studies. Nineteen attempted isolations showed that 12 of them were infected with the bacteria. A greater portion could have been obtained had the writers rejected the burrows made by very young larvae in unripe apple tissue.

#### Exit Holes

Exit holes in the fruit were commonly observed to show progressive decay in nature (Fig. 1, G, h). At this period the larvae had matured and worked out of the fruit to pupate in the soil. Spread of the rot about the exit holes progressed until the whole apple was decayed by *Phytomonas melophthora* or until other bacteria or fungi entered and completed this process in advance. Twenty-three apples were collected showing various stages of rot developing about exit holes. Positive cultures of the bacteria were secured from 11 of these fruits. Although all the exit holes showed decay, considerable difficulty was experienced in a number of cases owing to other bacteria and fungi. An unidentified fungus resembling *Mucor*

was particularly troublesome and was probably responsible for much of the failure to recover the apple-rot bacteria. The above experiments indicate that the larvae commonly enter the pupal stage carrying a considerable quantity of the plant pathogen.

### Puparia

Puparia (Fig. 1, H) were collected and stored in sand in the ice box for approximately 7 months. The purpose of storage was to try to determine

TABLE 1.—*Summary of isolation studies made on the association of apple-rot bacteria with different stages in the life cycle of the apple maggot and on the decay of apple tissue*

Source of isolation	Specimens employed	Specimens yielding pathogenic cultures	Isolations successful
	No.	No.	Per cent
Adult flies treated <sup>a</sup>	9	9	100
“ “ nontreated	16	12	75
Ovipositor puncture	22	10	45
Surface of eggs	15	8	53
Eggs from captive flies			
Treated <sup>a</sup>	2	0	0
Nontreated	3	3	100
Egg shells	24	8	33
Larvae from orchard			
Young larvae treated <sup>a</sup>	13	3	23
“ “ nontreated	13	3	23
Mature larvae treated <sup>a</sup>	13	13	100
“ “ nontreated	13	13	100
Larvae from captive flies			
Nontreated	6	6	100
From nontreated eggs <sup>ab</sup>	3	3	100
From treated eggs <sup>ab</sup>	2	0	0
Rot about larvae of captive flies	3	3	100
Larval burrows	19	12	63
Exit holes in fruit	23	11 <sup>c</sup>	48
Puparial surface washings	25	16	64
Puparia treated <sup>a</sup>	20	0	0

<sup>a</sup> The treatment was with a disinfectant, as described in the text.

<sup>b</sup> The eggs were removed from the apples and hatched in vials.

<sup>c</sup> The low number of positive results was probably because of secondary organisms, as explained in the text.

if time had a bearing on mode of overwintering of the bacteria. Twenty-five healthy puparia were chosen from the group and isolations made from surface washings. Ordinary methods of isolation failed in studies of the puparia, so certain modifications were employed as described earlier. Sixty-four per cent of these puparia were found to carry *Phytomonas melophthora* on that portion of the puparia that can be wet by water. These results indicate that overwintering in the field is likely to occur on the surface of the puparia.

Puparia, treated with disinfectants before crushing, gave no evidence of internal transmission of *Phytomonas melophthora*. Twenty puparia were used in these attempts, all of which gave negative results. Considerable difficulty was experienced with other bacteria and fungi that appeared in large numbers on the dilution plates. Cultures were secured from almost all of the various colonies appearing on these plates, and inoculations made into apple fruits gave negative results. The fact presented earlier that 1-day-old flies often carried the rot bacteria suggests that these bacteria were associated with the fly in the pupal stage. In this and earlier isolation studies it should be borne in mind that the rot bacteria, when present, might easily be missed, especially in badly contaminated material. The results of the above experiments suggest that *Phytomonas melophthora* may overwinter more abundantly on the surface of the puparia than inside.

#### SUMMARY

*Phytomonas melophthora*, the apple-rot bacterium, has been studied in relation to the various stages in the life cycle of *Rhagoletis pomonella*, the apple maggot. The bacteria have been found commonly associated with both male and female adult flies, eggs, larvae, and puparia. They have also been found in the ovipositor punctures, larval burrows, and exit holes in apple-fruit tissue. The bacteria were recovered from adult flies, and larvae, following treatment with surface disinfectants.

This work, based on 244 isolations, 54 per cent of which were positive, indicates that these apple-rot bacteria may be frequently associated with various stages in the life-cycle of the apple maggot.

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# STUDIES ON ALFALFA MOSAIC<sup>1</sup>

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A preliminary report on the occurrence of a transmissible virosis of the mosaic type affecting alfalfa (*Medicago sativa* L.) in California has been made previously.<sup>2</sup> As far as the writer has been able to learn, the existence of such a disease was not definitely proved prior to that time, although a few reports, evidently based on field observations, had been made. Miss J. I. Wood, of the Division of Mycology and Disease Survey, of the United States Department of Agriculture, in a letter to the writer, states that a mosaic of alfalfa has been reported from California, Montana, New York, Tennessee, Utah, and Washington. Dickson<sup>3</sup> reports that in one instance there appeared to be a successful transfer of mosaic from *Trifolium pratense* L. to *Medicago sativa* by means of aphids.

Elliott<sup>4</sup> attempted to infect alfalfa with the mosaics from sweet and red clovers but without success. Kunkel<sup>5</sup> lists alfalfa among the plants susceptible to a mosaic. F. R. Jones found what apparently is the same disease in Wisconsin and sent affected plants to the writer for study. While making an alfalfa-disease survey trip in 1928, what is thought to be the same disease was seen in a field near Twin Falls, Idaho. The disease also was found in Arizona in 1931. It seems probable that a careful survey would show the mosaic to be pretty generally distributed in many parts of the country. The purpose of this paper is to give a detailed description of the symptoms of the alfalfa mosaic previously reported<sup>2</sup> and to present experimental data to support the contention that the disease can be transmitted by aphids.

## SYMPTOMS

The first evidence of the disease in a leaf is the appearance of one or more small, more or less circular, greenish yellow spots. These areas frequently consist of a yellowish band of tissue,  $\frac{1}{2}$  to 1 mm. in width, surrounding an island of apparently normal color,  $\frac{1}{2}$  to 2 mm. in diameter. In some cases

<sup>1</sup> Cooperative investigations between the California Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

<sup>2</sup> Weimer, J. L. Alfalfa mosaic. *Phytopath.* 21: 122-123. 1931.

<sup>3</sup> Dickson, B. T. Studies concerning mosaic diseases. Macdonald College, Canada, Tech. Bul. 2. 125 pp. 1922.

<sup>4</sup> Elliott, J. A. A mosaic of sweet and red clovers. *Phytopath.* 11: 146-148. 1921.

<sup>5</sup> Kunkel, L. O. Virus diseases of plants. In Rivers, T. M., ed. *Filterable viruses*. P. 335-364. The Williams and Wilkins Co., Baltimore. 1928.



FIG. 1. A. Alfalfa leaves showing characteristic mosaic lesions. B. Branches of an alfalfa plant sent to the writer by F. R. Jones from Madison, Wisc. The spots on these leaves are smaller and the crinkling is somewhat more pronounced than is typical of the mosaic described and illustrated elsewhere in this article. This plant is thought to have been affected with a different mosaic. Both about  $\times \frac{1}{2}$ .

there are two or more concentric rings of green surrounding narrow bands of chlorotic tissue.

The chlorosis gradually spreads until eventually the rings are more or less obscured. There may be only one spot on a leaf, but more frequently there are several. These spots often coalesce, involving considerable areas of the leaf (Fig. 1, A). The affected tissue is greenish yellow at first, but gradually becomes lighter, as more chlorophyll is broken down, until it is distinctly yellow, and in extreme cases, especially where the leaf remains on the plant for a long time, almost white in color. The chlorosis may spread in all directions from the central ring so that a considerable amount of the tissue may eventually become involved. Although the rings may lie between or on the veins, the yellow color characteristically runs parallel to the veins and frequently involves them. Besides being chlorotic, affected leaves may be reduced to  $1/3$  their normal size, crinkled, and more or less deformed (Fig. 2), although this happens only in severe cases, usually in the spring before the first cutting when the disease is most conspicuous. In some leaves there is little evidence of ring formation, although there is considerable mottling. Severely affected leaf tissue often is considerably thickened and brittle, breaking easily when bent. There appears to be some dwarfing of the affected stems when the disease is severe, but, for the most part, there is no visible effect on the growth of the plant. No necrotic lesions have ever been seen on the stems. Only the lower leaves of a plant or all of them, including those not fully expanded, may be affected (Figs. 2 and 3). Diseased spots do not fall out, but remain intact throughout the life of the leaf.

The disease does not cause premature defoliation.

#### METHODS

At first several of the methods commonly used for transmitting different viroses were tried. Affected leaves were ground in a mortar, the juice obtained was placed on the young leaves of growing plants, and needle pricks were made through the juice into the tissue beneath. Likewise, leaves were crushed between the thumb and forefinger and then healthy leaves were rubbed, but only negative results were obtained. Several other mechanical methods were tried but with negative results.

A species of leafhopper, believed to be *Empoasca fabae* Harris, and one of thrips, probably *Euthrips occidentalis* Perg., both of which are common on alfalfa in southern California, although not positively identified, were tested as possible vectors, with negative results. Several experiments were then conducted in which aphids, *Illinoia pisi* Kalt., were used as vectors. These will be discussed in some detail.

The cages used in these experiments were similar to those commonly employed in insect transmission work. They consisted of a wooden frame, 9½



FIG. 2. Stem of an alfalfa plant all of whose leaves show mosaic symptoms. Note crinkling in some of the lower leaves. About  $\times 1$ .



FIG. 3. Stem of alfalfa plant with lower leaves showing numerous mosaic lesions and those at the top few or none. About  $\times 1$ .

by  $9\frac{1}{2}$  by 16 in. in dimensions, covered on 3 sides with cheese cloth (45 meshes to the inch). In the 4th side, or front, window glass was inserted. The 2 ends were of  $\frac{3}{4}$ -in. board, in the center of which were cut circular openings 6 in. in diameter. The sides and the hole in the top were covered with cloth, while the other end rested on the pot containing the plants. A thick layer of cotton, placed about the plants, extended over the edge of the pots to prevent the entrance of insects between the base of the cage and the pot.

Unless otherwise stated, the aphids (25 to 75) were transferred from the breeding cages, where they were feeding on plants affected with mosaic, to the healthy plants, likewise growing under cages, by sucking them into a glass tube thrust through a hole in the cloth side and then blowing them into the cage enclosing the healthy plants. The opening in the cloth was kept tightly plugged with cotton when not in use. In Experiment 454 the aphids were shaken from twigs, taken from breeding cages, directly upon the healthy plants, and in Experiment 458 the same procedure was followed except that the aphids were first caught upon paper and then transferred to the plants. In both cases care was exercised to exclude other insects that might possibly have been in the breeding cage. The aphids were allowed to feed on the inoculated plants for from 48 to 72 hours and then were killed by spraying the plants thoroughly with nicotine and soap. The cages used did not exclude insects as small as thrips, either from inoculated or the control plants, but, since the control plants never developed mosaic, the presence of these insects was not considered important. The aphids in the breeding cages were collected originally from alfalfa growing in the field. Those in the original collection were identified by Ralph H. Smith, Entomologist of the University of California, as the pea aphid (*Illinoia pisi* Kalt.) and subsequent collections were assumed to be the same. Alfalfa plants of the Chilean variety growing in mountain subsoil in 8-in. pots were used throughout. For the most part, the seedlings were inoculated when about 2 months old. Controls, consisting of plants of the same lot held under cages, but without the addition of aphids, were run in all the experiments and in no case did any of these become infected.

No mosaic symptoms ever developed as the result of the feeding of aphids on the plants in those experiments in which negative results were obtained. Likewise, alfalfa suffering from heavy aphid infestation has been observed in many localities without mosaic symptoms appearing. Once a plant is infected with mosaic, the symptoms may reappear after each cutting, even though the plant be kept entirely free from insects. This recurrence of symptoms would not occur if the trouble were due directly to the insects feeding upon them. These facts convinced the writer that aphids were not themselves responsible for these mosaic symptoms, but only served as vectors, hence controls in which noninfective aphids were placed on healthy plants were deemed unnecessary.

## EXPERIMENTAL DATA

The data obtained in 6 experiments are given in table 1. The dates on which the aphids were placed upon the plants and those on which the first symptoms were observed are shown in the second and third columns. The difference between these dates in each experiment, usually 7 to 14 days, denotes the approximate incubation period. There appears to have been an unusually long incubation period in experiment 421. No explanation for this can be given. It may be stated, however, that the seedlings in this experiment were younger when inoculated than those commonly used, having only their first true leaves. The temperature for some time preceding the discovery of the first symptoms was so high that no mosaic was evident in the uncovered plants in near-by pots or in the field. Just prior to July 22 there were several foggy days, which resulted in the lowering of the daily maximum temperature by several degrees, and mosaic had reappeared in the plants in the field and in pots on this date.

Usually, several alfalfa seedlings were growing in a pot, and the tops soon became so intermingled that accurate counts were difficult to make, especially since these plants were always covered by cages. Rather than remove the cages and expose the plants to insect attack while the stems were being untangled and the plants counted, it was decided to make as accurate an estimate as possible without removing the cages. For this reason, the numbers of plant given in the table, where more than one or two are involved, are only approximately correct. The figures obtained show that out of a total of 74 plants 27, or 36.5 per cent, became infected.

TABLE 1.—*The results of inoculating alfalfa plants with mosaic, using aphids as vectors on six different dates in 1930*

Experi- ment number	Date inoculated	First symptoms apparent on	Approxi- mate number of plants inoculated	Approxi- mate number of plants infected
417	March 20	April 2	2	1
421	April 7	July 22	20	5
422	April 10	April 18	20	2
454	Sept. 27	Oct. 4	10	10
455	Oct. 1	Oct. 13	2	2
458	Oct. 11	Oct. 21	20	7

## DISCUSSION

In those plants that became infected in these experiments, mosaic symptoms usually appeared first in one or at most only a few of the leaves present

when the inoculations were made, but those leaves that developed subsequently commonly showed infection. Although not so high a percentage of infection was obtained in some experiments as might have been expected or desired, yet the data presented indicate pretty conclusively that alfalfa mosaic can be transmitted by the pea aphid. It is thought that a higher percentage of infection would have been obtained had the experimental conditions been more favorable for the development of the disease. As no greenhouse space was available, the experiments were all conducted outdoors. It was very difficult to find a time when all conditions were suitable. For example, when the plants were in the proper stage of growth for inoculating, the temperature might be too high or too low, or when the temperature was right the plants were not. That temperature is a factor in the appearance of mosaic symptoms, and probably in infection also, is suggested by the fact that the disease is not evident in plants growing in the field during either hot or cold weather, but may be very conspicuous in 100 per cent of the plants during moderately cool periods, such as occur in the spring and autumn months, or in the summer during prolonged foggy periods. Light also may have been a factor in the appearance of the mosaic symptoms. It will be noted in table 1, that infection was obtained in March, April, September, and October. Similar experiments conducted in December, January, February, and May gave negative results.

The plants used in some of these experiments were not in the best condition for infection with mosaic or for its continued appearance, since they did not do well under cages and the leaves often became badly affected with a leaf-spot (*Pseudopeziza medicaginis* (Lib.) Sacc.) or with rust (*Uromyces medicaginis* Pass.).

It is possible that other insects will be found to transmit the disease more readily than the species of aphids used. That the disease is insect-transmissible is indicated also by the fact that plants held under cages for an indefinite period remained free of mosaic, while other plants to which insects had free access usually became diseased when quite young. That the virus is perennial in the tissue was shown by keeping two affected alfalfa plants caged for 2 years. The tops were cut off several times during this period and the new leaves always had more or less mosaic. No evidence that the disease is present in the seed or soil was obtained. Although over 100 different strains and varieties of alfalfa have been under observation, no indication of resistance has been seen in any of them.

F. R. Jones, in a letter dated June 30, 1930, stated that he also apparently had obtained a small percentage of infection by placing aphids from alfalfa plants affected with mosaic onto healthy plants.



## A POSSIBLE SECOND VIROSIS IN ALFALFA

As already mentioned, F. R. Jones sent a number of alfalfa plants affected with mosaic from Wisconsin to the writer for comparison with the California strain. All of these plants, with one exception, exhibited mosaic symptoms identical with those of similarly affected plants in California. The leaves of the excepted plant were more crinkled and the spots were very small and lacked the characteristic rings. The symptoms of this disease are shown quite well in the picture of 2 of the twigs from the plant in figure 1, B. The general appearance of the plant suggested the presence of a virosis of the mosaic type but different from the mosaic discussed in this paper. A single unsuccessful attempt was made to transmit the disease by means of aphids. In a letter accompanying the plant, Dr. Jones stated that it had grown near many other kinds of plants affected with mosaic. It seems probable that some other virus had been transmitted to this alfalfa plant. Field observations have suggested the existence of still other mosaic-like viruses capable of attacking alfalfa.

## SUMMARY

A transmissible virosis of alfalfa, of the mosaic type, is described and illustrated. The disease has been transmitted by means of the pea aphid. The existence of what appeared to be a slightly different type of mosaic on alfalfa also is noted.

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# OCCURRENCE OF TOBACCO RING-SPOT-LIKE VIRUSES IN SWEET CLOVER<sup>1</sup>

R. G. HENDERSON

(Accepted for publication May 24, 1933)

## INTRODUCTION

Earlier reports (1, 2, 6) have indicated that there may be some connection between the occurrence of ring spot in the tobacco crop and the growing of sweet clover in tobacco rotations, or to the presence of sweet clover in the vicinity of tobacco fields. Such a relationship is indicated by the fact that ring-spot infection is often very severe in tobacco following a sweet-clover crop. No experimental work, however, has been done to determine whether the sweet clover is directly responsible for this increase in the amount of disease.

## REVIEW OF LITERATURE

Wingard (6) reported that sweet clover, *Melilotus officinalis* Lam., was susceptible to the tobacco ring-spot virus, and that it occurred naturally on sweet clover. The natural occurrence of the tobacco ring-spot virus on sweet clover has been reported also by Henderson and Wingard (2). Fenne (1) stated that typical ring spot of tobacco was obtained from sweet clover, *Melilotus alba* Desr., on several occasions. Young<sup>2</sup> reported 30 per cent infection of white sweet-clover plants with ring spot in a one-fourth acre field in Montana.

## OCCURRENCE OF RING-SPOT-LIKE VIRUSES ON SWEET CLOVER

It is difficult to estimate the extent of the natural occurrence of viruses on sweet clover that will produce symptoms on tobacco similar to ring spot. Such virus-infected sweet-clover plants cannot always be positively identified by symptoms, alone. Plants have been collected exhibiting symptoms that were presumed to be caused by the ring-spot virus, but, when such material was used as inoculum on tobacco, no infection was obtained. Abnormal sweet-clover plants, however, have been collected in various parts of Virginia that were definitely infected with a type of ring-spot virus. The inoculation of tobacco plants with juice from these abnormal plants has resulted in the production of ring-spot-like symptoms. At first, the viruses obtained from sweet clover were considered identical with the tobacco ring-spot virus, but

<sup>1</sup> Paper No. 81 from the Department of Botany and Plant Pathology, Virginia Agricultural Experiment Station.

<sup>2</sup> Young, P. A. Ring spot of white sweet clover in Montana. U. S. D. A. Bur. Pl. Ind. Pl. Dis. Repr. 14: 125. 1930.

a closer examination of the symptoms produced on tobacco by these viruses indicates that they may be different from it. Henderson and Wingard (2) stated that the only significant difference between the sweet-clover viruses and the tobacco ring-spot virus was in the intensity of the symptoms produced on tobacco. The viruses from sweet clover seemed to be attenuated forms of the tobacco ring-spot virus. In some instances inoculations on tobacco with a sweet clover extract produced primary lesions that could have been considered identical with primary lesions of ring spot, but the secondary symptoms produced by systemic infection proved to be very mild. In most cases the sweet-clover virus was lost in subsequent transfers. The illustrations shown in the previous publication (2) were of a disease on tobacco caused by a sweet-clover virus that appeared to be less attenuated than the majority of the other viruses obtained from this host.

Most of the observations on sweet clover have been made when the plants were immature; therefore, it was not always possible to determine whether they were of the yellow or white species. Wingard's work was with the yellow species (*Melilotus officinalis*), but in all probability the majority of the specimens collected on different farms by the writer were of the white species (*M. alba*), since it is the one commonly used commercially in Virginia.

The ring-spot-like virus on sweet clover appears to be in a more virulent form during early spring; in fact, the virus seems to become inactivated in the summer. Sweet clover plants collected on May 2, 1932, were infected with the new virus disease described below. Additional plants collected about 3 weeks later in the same locality and exhibiting similar symptoms did not carry an infectious virus as shown by inoculations on healthy tobacco. In other instances the writer has been unable to obtain infection on tobacco in midsummer from sweet-clover plants that showed the usual symptoms of virus infection.

#### A NEW VIRUS ON SWEET CLOVER

In early May, 1932, a specimen of sweet clover was collected near Winchester, Virginia, that appeared to be affected with a virosis. The leaves showed chlorotic markings of various shapes (Fig. 1, B and C). The chlorotic areas often followed the midrib of the leaflets and extended along the lateral veins towards the margin. Some leaflets showed chlorotic blotches and spots of various sizes. Very often the markings were of fine lines that followed along the veins or ran in irregular circles. The younger leaves were severely dwarfed and deformed with much puckering and twisting of the bud leaves. This characteristic is noticeable in the two specimens on the left in figure 1, D. The specimen on the right in this picture is from a healthy plant. Figure 1, A, is a leaf from a healthy plant.



FIG. 1. Sweet clover showing symptoms of the new virus disease. A. Healthy leaf. B and C. Leaves showing chlorotic markings. D. Shoots showing dwarfing and distortion of leaves as compared with healthy specimen on right.

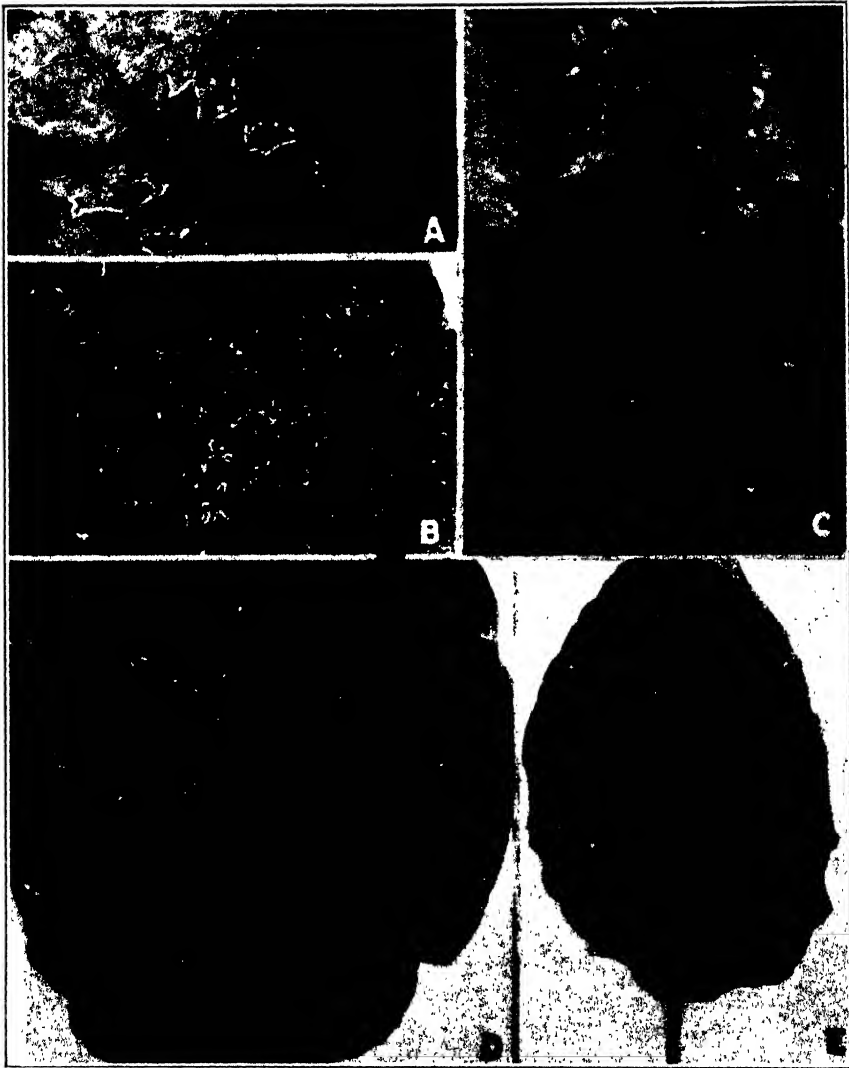


FIG. 2. Symptoms on Turkish tobacco produced by the new virus from sweet clover. A. Portion of leaf showing necrotic zigzag lines (slightly enlarged). B, D, and E. More severe forms of symptoms. C. Faint chlorotic markings on young leaf without the presence of necrotic lesions.

#### Inoculation of Tobacco

Leaves taken from the abnormal sweet-clover plants were ground in a mortar with a little water and used to inoculate healthy Turkish tobacco plants in the greenhouse. A few faint rings or spots appeared on the in-

oculated leaves in about 6 days. A little later systemic infection developed and it was observed that the symptoms were quite different from those that had been obtained from previous inoculations with sweet-clover extracts. The first noticeable expression of systemic infection was a few faint chlorotic or necrotic zigzag lines following, more or less, some lateral vein (Fig. 2, A). Still fainter contour-like lines could be seen in the tissue when the leaf was held to the light. By very close examination these "contour" marks can be seen in figure 2, A and C.

On certain leaves the symptoms became more pronounced as shown in figure 2, B, D, and E. The nature of these symptoms varied considerably. Chlorotic or necrotic lines followed along the veins on some leaves. On other leaves the lines were in the form of rings, or followed the smaller veins, cutting off the intercostal tissue into islands of irregular shape. On some of the more severely affected leaves the tissue along the veins was a darker green than the rest of the leaf. A part of the leaf in figure 2, E, shows this symptom.

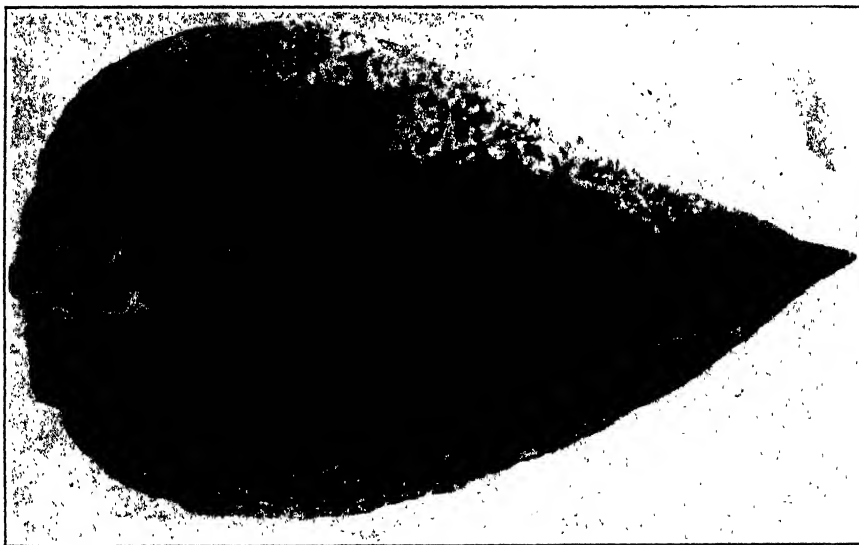


FIG. 3. Leaf of Turkish tobacco showing chlorotic or bleached lines and blotches produced by the new sweet-clover virus.

At times broad chlorotic or bleached lines appeared that were unlike those described above. (Fig. 3.) These bleached lines followed the veins, more or less, and were usually near the leaf margin. Yellow blotches also were present on the central part of the leaf. This bleached type of symptom usually appeared on the upper leaves and was first noticed on leaves that nearly touched the glass roof of the greenhouse in which the plants were growing.

It is of interest to note that the symptoms described above did not occur on all of the leaves. Some of the leaves apparently were healthy, while others, immediately above and below them, showed symptoms. Tobacco plants affected with this virus from sweet clover did not appear to mask the symptoms

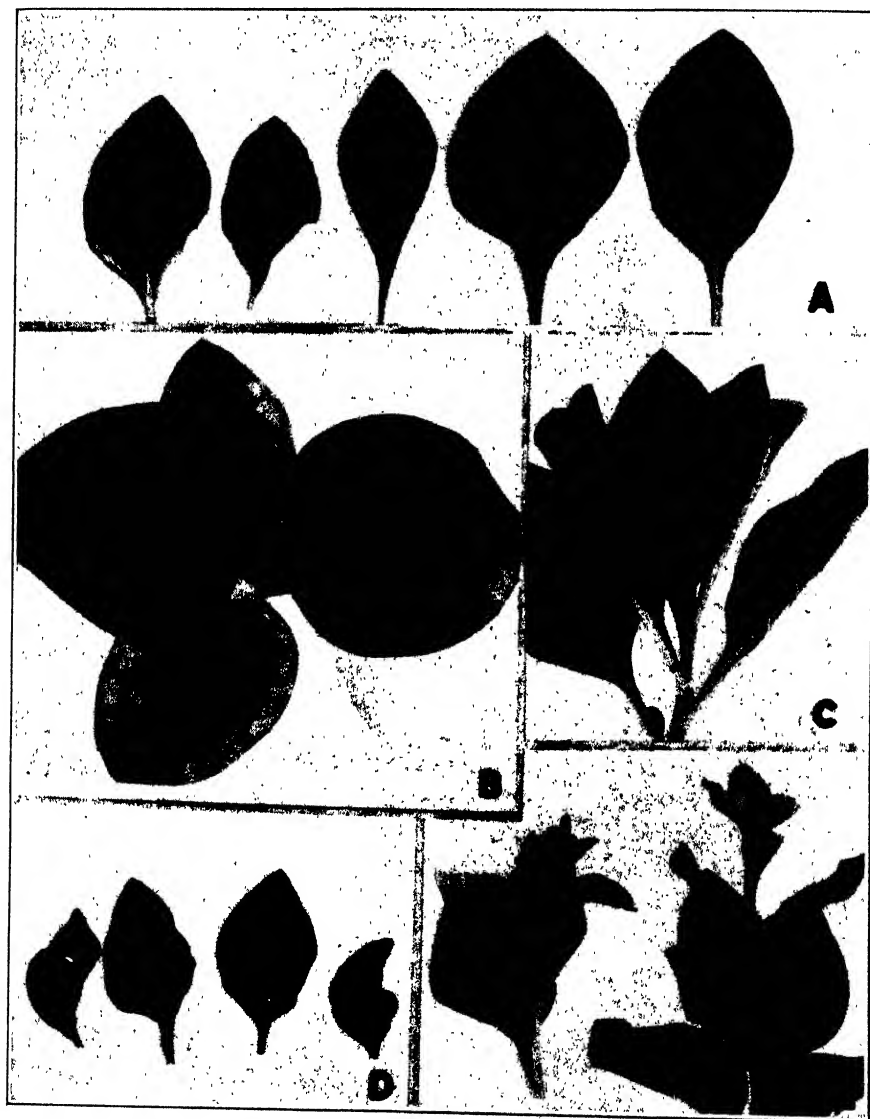


FIG. 4. Symptoms of sweet-clover-virus infection on petunia. A and B. Leaves showing chlorotic streaks and blotches. C. Shoot from healthy plant. D. Leaves from diseased plant showing more severe or necrotic type of symptoms. E. Shoots from diseased plants showing necrotic lesions on bud leaves.

permanently or develop an immunity from it, such as that described for true tobacco ring spot by Wingard (6) and also by Price (4).

### Inoculation of Petunia

Healthy petunia plants were inoculated with the clover virus. No signs of infection were noticed on the inoculated leaves, but in about 10 days symptoms began to appear on the new leaves. The leaves were chlorotic in areas along the margin and in spots and blotches in other parts. (Fig. 4, A.) On some leaves pale green areas extended along the midrib and out between the veins. The whole leaf in some cases became yellow to light green with the exception of a few dark green spots or streaks near the base. In other cases the chlorosis was in the form of small specks along the midrib and veins or over the entire leaf, giving it a gray cast. At times the chlorosis gave the foliage a variegated appearance. (Fig. 4, B.)

The symptoms on petunia did not vary so much as they did on tobacco. None of the leaves appeared to be free of the disease, as was the case with tobacco. Every leaf showed some chlorosis. At times the disease became quite severe on the tips of certain branches. The leaves were dwarfed and deformed. Spots and streaks in the tissue died (Fig. 4, D and E). Figure 4, C, shows a healthy branch of petunia for comparison.

### Inoculation of Sweet Clover

White sweet-clover plants growing in the greenhouse were inoculated with expressed juice from tobacco infected with the sweet-clover virus. The results, however, were negative. The inoculations were repeated, but no signs of infection appeared. Thinking that possibly the symptoms were masked or had been overlooked, inoculations were made from the sweet-clover plants back to tobacco, but the results were negative, showing that the plants had not become infected.

The writer has also been unable to obtain infection of white sweet clover with the true tobacco ring-spot virus. As stated above, Wingard obtained ring-spot infection on yellow sweet clover. It would seem from this that there was a difference in the susceptibility of the yellow and white species. The writer believes, however, that his failure to secure infection of sweet clover was probably due to some environmental factor. In work to be reported later, it has been determined that ring-spot infection on tobacco is very greatly influenced by the air temperature in which the plants are growing. It is probable that temperature also is a factor in sweet-clover infection. P. A. Young,<sup>3</sup> who reported natural occurrence of ring-spot on sweet clover in Montana, further reported that he was able to transfer the virus from sweet clover to bean but attempts to inoculate healthy sweet-clover plants were unsuccessful.

<sup>3</sup> In personal correspondence to the writer dated April 5, 1933.



## DISCUSSION

The question of the ring-spot disease of tobacco has become more complicated with the discovery of viruses on other host plants that are able to infect tobacco and produce a disease with symptoms very similar, yet not identical, to ring spot. In only a few cases has the tobacco ring-spot virus been positively identified as occurring in nature on other hosts. With the wide distribution of ring spot in tobacco and the occurrence of such a high percentage of infected plants in certain fields, there seems to be some indication that other hosts carry the virus over winter. Evidence so far points to sweet clover as an over-wintering host plant. Nevertheless, it has not been proved that the ring-spot-like viruses occurring in sweet clover are identical with the tobacco ring-spot virus. The relation of the viruses from sweet clover to the tobacco ring-spot virus, therefore, remains to be determined. Johnson (3) and Vallean (5) have described a number of viroses of tobacco that somewhat resemble ring spot. It is clear, however, that the symptoms of the various diseases are not the same and also that the properties of the viruses differ in many respects. These diseases have been considered by Johnson and Vallean as being caused by separate and distinct viruses. On the other hand, the symptoms that the writer has obtained on tobacco by inoculations with viruses from abnormal sweet clover plants have all been similar, though not always identical, and in one case they have been identical (as to virulence) to the symptoms of true tobacco ring spot. It would seem more plausible to classify these sweet-clover viruses as strains (with possibly some exceptions) of one virus rather than consider them as separate viruses.

Failure to obtain infection by artificial inoculation of sweet clover with the ring-spot and ring-spot-like viruses has handicapped this phase of the work. A virus can be transferred from diseased sweet clover to healthy tobacco without difficulty, but the writer has not succeeded in transferring the virus from diseased tobacco back to healthy sweet clover. The environmental conditions necessary for the virus to infect sweet clover seem limited to a narrow range.

It is well known that environment influences the susceptibility of certain plants to diseases. For example, it is extremely difficult to get ring-spot infection on *Nicotiana glutinosa* L., but under certain conditions, which have not been determined, infection of this plant with ring spot can be secured. It is possible that if the proper environmental conditions prevail, infection can also be obtained on sweet clover with the ring-spot virus.

## SUMMARY

The natural occurrence of ring-spot-like viruses on sweet clover is discussed. Viruses of the ring-spot type have been found to occur on sweet

clover in various parts of Virginia. It is suggested that the viruses from sweet clover may be forms or strains of the tobacco ring-spot virus.

A new virosis of sweet clover is reported and the supposed symptoms described. This virus is infectious on tobacco, producing symptoms of the necrotic-pattern type but distinctly different from the symptoms of ring spot. It is also infectious on petunia, producing a type of chlorosis. The virus was not successfully transferred back to healthy sweet clover plants.

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# YELLOW RUST OF RUBUS<sup>1</sup>

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(Accepted for publication April 24, 1933)

A general description of the yellow rust of *Rubus* has been presented in a previous paper (6) in which the causal organism was considered to be *Phragmidium imitans* Arthur. In our consideration of the taxonomy of the species since then, the question of its identity with the European *P. rubi-idaei* (DC.) Karsten has repeatedly arisen. Grove (4, pp. 298-299) briefly mentions *P. imitans* as very similar to the European species, having similarly formed aecia and pycnia.

In order to obtain more direct evidence on this relationship, materials from America and Europe were carefully compared. Thanks to the courtesy of Dr. E. J. Butler, dried specimens of uredia and telia of *P. rubi-idaei* were secured through the British Imperial Mycological Institute. These had been collected in August, 1931, from the wild raspberry, (*Rubus idaeus*) in England. Briosi and Cavara Exsiccate No. 130,<sup>2</sup> in the Herbarium of Oregon State College, supplied aecial material from Italy, to compare with Oregon specimens. We have been able to detect no differences between this European and Oregon material and have concluded that the Oregon rust is identical with the European and should bear the older name, *Phragmidium rubi-idaei* (DC.) Karst. The same conclusion was reached by Dr. J. C. Arthur, according to a recent communication.

The present study (1) has aimed to follow the complete life history and morphology of the rust by means of infection from artificial inoculation in the greenhouse and natural infections under field conditions, (2) to determine from greenhouse and field observations the relation of certain ecological factors to infection and epidemics of the disease, and (3) to compare the susceptibility of certain species and varieties of *Rubus* to the disease.

## LIFE HISTORY AND MORPHOLOGY

Since the Cuthbert red raspberry is known to be very susceptible to yellow rust, this host plant was used for inoculation studies in the greenhouse and for observations concerning the life history of *Phragmidium rubi-idaei* in the field. Inoculations in the greenhouse were made in a tent-like humidifier where a moisture-saturated atmosphere could be maintained.

<sup>1</sup> Published as Technical Paper No. 197 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

<sup>2</sup> Briosi, G., and F. Cavara. I Funghi Parassiti, Exsicc. Fasc. VI: No. 130. Pavia. 1891.

Potted plants to be inoculated were previously placed in the humid chamber for 12 hours so that the leaves were in the best possible condition for infection. Then spores of the desired stage were dusted or brushed on certain marked leaves and the plants allowed to remain under the moist conditions for 72 hours after inoculation. Then they were returned to ordinary greenhouse conditions. The work began with teliospore germination and sporidial infections in the greenhouse.

*Teliospores and Their Germination.* Teliospores first appear under field conditions early in July and increase in quantity during the summer, even through very dry weather. By late September or early October the telial stage is the only one to be found, the lower surface of infected leaves having a sooty black cast due to the abundance of teliospores. If the raspberry leaves fall undisturbed they alight bottomside up, exposing a maximum number of the telia—a very important natural behavior in the economy of this stage of the rust.

The teliospores of *Phragmidium rubi-idaei* require a considerable rest period before they will germinate in quantity. Several attempts to germinate them in hanging drops failed early in the autumn, even after exposure to freezing temperatures, and the first successful germinations were secured on living leaves in the greenhouse during January, although previous attempts had failed. Simultaneously, difficulty was experienced in obtaining germination in water. Evidently, the host exerts a stimulating influence on teliospore germination, and the conditions affecting our water-germination experiments did not simulate closely enough the natural conditions prevalent from March to May, when sporidial infection normally takes place.

From greenhouse inoculations with teliospores it was discovered that pycnia make their first appearance in 10 to 13 days after the spores were placed on the leaves. Under field conditions the first leaves of the Cuthbert red raspberry unfolded at the base of some marked fruiting canes on March 15. On March 25 and 26 pycnia were first recognized on the lower leaves of these canes, in close proximity to teliospore masses on old leaves that had over-wintered on the ground, and were especially abundant where dead leaves had accumulated. The first infection period was accompanied by a period of prolonged wet weather and moderate temperatures, after which pycnia and aecia began to appear.

A histological and cytological study of the rust through its complete life history was carried out by the junior author<sup>3</sup> who found the same story

<sup>3</sup> This histological and cytological study, along with the field and host susceptibility studies presented in this paper, comprised a subject assigned to the junior author for a thesis in partial fulfillment for the degree of Master of Science, June, 1932. This complete thesis, with full data, is filed in the Library of Oregon State Agricultural College, Corvallis.

for *P. rubi-idaei* as that presented by Blackman (2) for *P. violaceum* and by Christman (3) for *P. speciosum*, except that in these species the pycnia are on the opposite side of the leaf from the aecia, while in *P. rubi-idaei* the aecia immediately surround the pycnia in a sorus that completely encircles them (4, 6). Whether the aecia and pycnia arise from the same mycelium is a question, but in the early stages of aecial development the primordial mycelium and buffer and stalk cells are uninucleate. It appears that the first binucleate cells occur in the erect aecial hyphae.

#### RELATION OF MOISTURE AND TEMPERATURE TO WAVES OF INFECTION

The climatic factors influencing spore germination and leading to epidemics of the various stages of yellow rust are worthy of brief consideration. Among other climatic factors, moisture and temperature are evidently most responsible for waves of aecial and uredial production, moisture being the prime factor. Our records show that any climatic condition, such as rainfall and fog or other forms of very high humidity, which result in the formation of films of free water on leaf surfaces, is conducive to infection by yellow rust, provided these moisture conditions continue for several hours at a time.

In order to study the progress and numerical intensity of the occurrence of aecia and uredia during the spring and summer of 1931, 24 noninfected leaves were labeled. At each interval of 7 days these leaves were carefully observed for the first appearance and progress of rust infections. By this method it was found that infection took place during moist weather. With each repetition of such weather, the number of infections increased to a maximum and then, with drier weather, infection abruptly ceased. At each of the intervals at which infection ceased, new sets of noninfected leaves were labeled and observed for infection. During the period of investigation in 1931, the weather conditions resulted, therefore, in an intermittent series of 2 aecial and 2 uredial waves of infection. (Fig. 1.)

Figure 1 is a graphic presentation of the relative humidity, rainfall in inches, and temperature, correlated with the progress of waves of aecial and uredial production in terms of the average number of new infections. The climatological data used in this study are those taken by the U. S. Weather Bureau at 5 p. m. daily at Corvallis, Oregon. After the first appearance of pycnia on March 25 there was a steady increase in the number of aecia, which reached a maximum on May 7. This increase of aecia followed immediately after the more or less constant rainfall from March 6 to April 14. It constituted the major infection wave that year and definitely established rust in the fields. The next wave of aecial infection started about May 15 and reached a maximum about June 3. This was not so severe as the first wave, undoubtedly because of the generally lower level

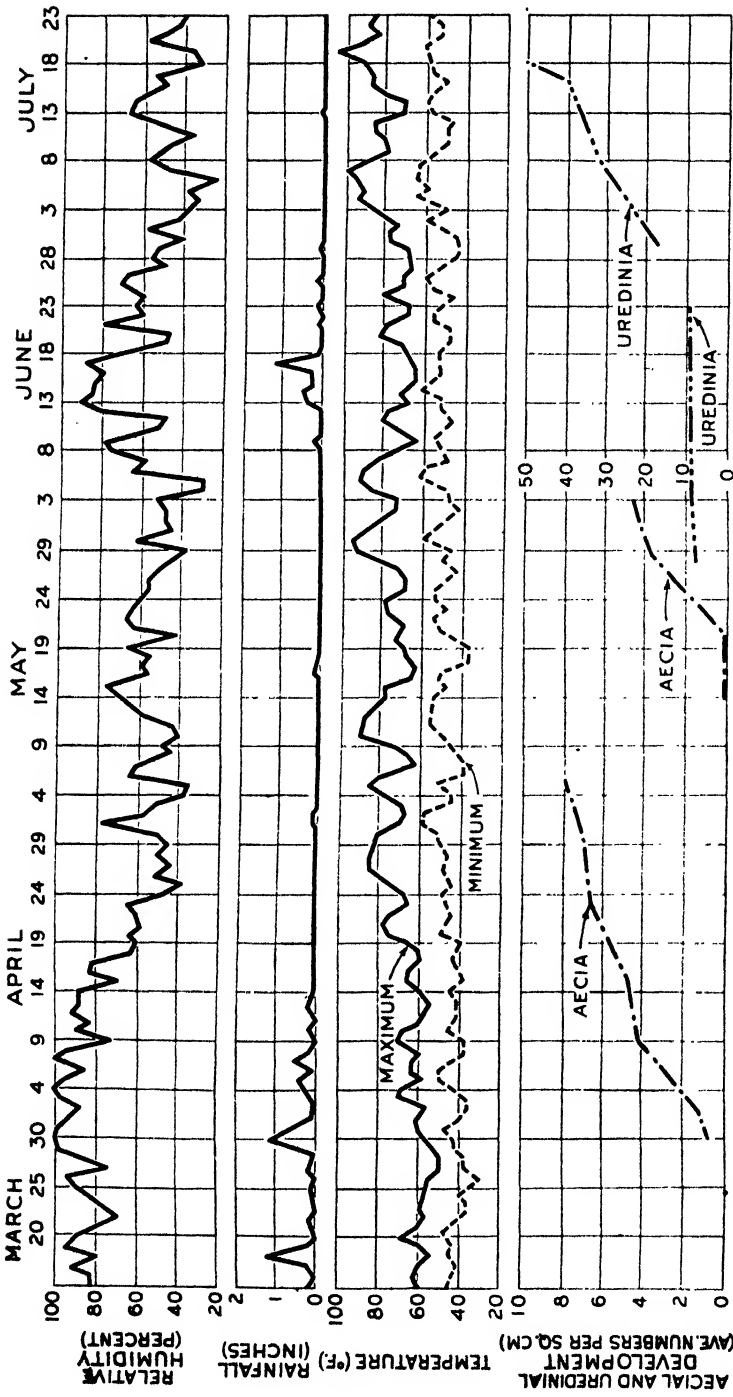


Fig. 1. Relation of precipitation, relative humidity, and temperature to waves of aecial and uredinal production during the spring and summer of 1931, Corvallis, Oregon. The development of aecia and uredia is expressed in terms of the average number of new infections per square centimeter of leaf surface.

of relative humidity and less rainfall, accompanied by somewhat higher temperature during this second period. The slight drop of temperatures from May 15 to 27 accompanied by rainy weather during May 16 to 18, inclusive, was conducive to aecial production, which followed into June, when uredial sori first became noticeable.

The uredia very gradually increased from late May to mid-June but the one major wave of infection resulted from the slightly lower temperatures and higher humidities accompanying the rains from June 8 to 29. This wave of uredia started in earnest about June 28 and reached a maximum about July 21 (Fig. 1). Relatively high humidity, cool temperature, and slight precipitation from July 12 to 15 and, again, from September 5 to 10, produced rather continuous but slight waves of uredia following both periods.

Figure 1 shows graphically the more conspicuous waves of aecial and uredial infection during the spring and early summer of 1931. In the summer of 1932 there was rainfall each month. Each rainy period was followed by rapid increase of the prevailing stages of the rust and resulted in a cumulative severity of infection throughout western Oregon, greatly surpassing that of 1931.

Sporidial infections on Cuthbert raspberry in the greenhouse were brought about in 2 ways, by maintaining a moisture-saturated atmosphere in a closed chamber or by spraying moisture on the leaves in a humid chamber. Free water on the leaf surface is more readily maintained with a slight drop in temperature and high humidity. Under these conditions, mature aecia appeared in 10 to 14 days after inoculation, initial stages appearing in 6 to 7 days.

Inoculations made on the upper surfaces of certain leaves and on the lower surfaces of others reveal the fact that sporidia apparently infect one surface as readily as the other, irrespective of the number of stomata. Stomata are rarely found on the upper surface of Cuthbert raspberry leaves, but aecia are usually found more abundantly on the upper side under field conditions. Undoubtedly, under natural conditions the sporidia are for the most part borne by air currents in such manner as to alight on the upper surface of leaves, since greenhouse experience has shown that the aecia appear on the surface inoculated with sporidia.

Further, greenhouse inoculations with aeciospores and urediospores have shown mature uredia resulting in 8 to 13 days after inoculation, provided the same environmental conditions necessary for infection by sporidia were maintained. There is, however, one essential difference. Uredia always appear first directly below stomata on the lower surface of the leaf. All inoculations by aeciospores and urediospores on the upper surface of leaves were unsuccessful. These infections always take place through stomates.

Thus, by correlating the greenhouse experiments with the field observations shown in figure 1, it is readily seen that under natural conditions the lag of the waves of infection after a rainy period is doubtless due to the incubation period from incidence of natural inoculation until infections become apparent; also, that rainfall, in itself, supplies free moisture on leaf surfaces or is usually accompanied by slight drop in temperatures and sufficiently high humidities to bring about the necessary free moisture from fog or dew. These are pertinent facts in the prediction of epiphytotics of the rust caused by *Phragmidium rubi-idaei*.

#### SUSCEPTIBILITY

The Cuthbert raspberry is the one standard commercial red variety grown in the Pacific Northwest. The crop from this variety has several commendable qualities, but the plants, besides their susceptibility to *P. rubi-idaei*, lack other desirable characteristics. Consequently, other varieties are being tested for this locality and new hybrids are now being produced in an effort to find a suitable stock to replace or supplement the Cuthbert. For this reason it was desirable to procure information concerning the susceptibility of available varieties and species of *Rubus* that might be introduced or used as parents in the production of new hybrids.

Previous references to the disease have reported it in America on *Rubus leucodermis* Doug. (Western wild black raspberry), varieties of *R. occidentalis* L. and *R. strigosus* Michx., and the Salmon berry (*R. spectabilis* Pursh) (1, 6).

In Europe (4) *Rubus idaeus* L. and some of its horticultural varieties are more or less infected. In Saccardo (5) it is reported on *R. strigosus*, *R. idaeus* and *R. odoratus* from many European countries.

In the fall and winter of 1931-32, the following several species and varieties of *Rubus* were inoculated to determine their relative susceptibility to *Phragmidium rubi-idaei*: *Rubus laciniatus* Willd. (Evergreen blackberry), *R. leucodermis* Doug. (Western wild black raspberry), *R. loganobaccus* Bailey (Loganberry), *R. macropetalus* Doug. (Northwest trailing blackberry or dewberry), *R. parviflorus* Nutt. (Thimble berry), and *R. spectabilis* Pursh (Salmon berry). Of the red varieties used, the Antwerp and Lloyd George are supposed to be true horticultural varieties of *R. idaeus* L. and Ranere (St. Regis) a true variety of *R. strigosus* Michx. The probable hybrids of these two species of red raspberries tested include Cayuga, Chief, Cuthbert, Golden Queen, Herbert, Latham, Marlboro, Owasco, and Seneca. A purple variety, Webster, and 3 horticultural varieties of *R. occidentalis*, Cumberland, Munger, and Plum Farmer, were used. Three local species of wild rose, *Rosa gymnocarpa* Nutt., *R. nuthana* Presl., and *R. rubiginosa* L. also were tested to learn whether the yellow rust crosses over to this genus.



Potted plants of all these varieties and species were grown and inoculated in the greenhouse. Ten to 12 varieties were inoculated at a time under identical conditions. In each test the Cuthbert was included as a standard of comparison between the various groups tested. The inoculations were made in humidity chambers, where conditions favorable to infection were constantly maintained. Urediospores, continuously cultured in the greenhouse on Cuthbert raspberry, were used in all the inoculations. After inoculation the plants were incubated for 72 hours in the humid chambers. After removal 6 examinations were made and data taken at the end of 13, 15, 19, 23, 28 and 32 days from the time of inoculation.

After all varieties had been tested once they were divided into susceptible and resistant groups based on the first test. Each group was then tested again in duplicate. In every case the results of the second duplicate tests conformed with and bore out the findings of the preliminary experiments.

The number of infections per unit area of leaf surface was determined by actual count and the area estimated by the well-known method of matching leaflets with leaf-shape cardboards of known area. It was found necessary to take another factor into consideration when estimating the damage to the host. The uredial pustules were much larger on some varieties of *Rubus* than on others. Since the size of the pustule seems to indicate to some extent the degree of vigor of the rust on a particular host, this size factor was included in the estimate of degree of susceptibility of one plant compared with another.

The results of this inquiry into the susceptibility of host plants to infection by *Phragmidium rubi-idaei* can be summarized briefly. The species and varieties found immune from yellow rust included all of the blackberries tested, *Rubus parviflorus*, *R. spectabilis*, the Munger and Plum Farmer varieties of *R. occidentalis*, and the three roses *Rosa gymnocarpa*, *R. nutkana*, and *R. rubiginosa*.

All infection counts and general characters brought about by infection of the varieties showing degrees of susceptibility are given in table 1. The numbers indicate infections per square centimeter of leaf surface and the letters refer to the comparative sizes of the pustules, L indicating large, M medium, S small, and VS very small. On the 28th day after inoculation telia were observed. The column designated telia refers to their degree of abundance, H indicating heavy, M medium, S slight, and N none. The varieties are placed in the order of their susceptibility as arbitrarily determined by the intensity of infection and the size of the uredia.

The significant results presented in this table, so far as desirable red raspberries are concerned, are the extreme susceptibility of the Cuthbert and Marlboro, and mere disease tolerance of the Lloyd George. It is of

TABLE 1.—*Study of relative susceptibility of certain varieties and species of Rubus as indicated by greenhouse tests*

Host	Period after inoculation					
	13 days	15 days	19 days	23 days	28 days	32 days
	Number of infections per sq. cm. of leaf surface				Telia	Condition of plant
Marlboro	12.4 L	19.4 L	65.6 L	80.8 L	S	Heavy infection; 100% of spots necrotic.
Cuthbert	7.6 S	16.8 L	39.9 L	67.8 L	II	Heavy infection; 100% of spots necrotic.
<i>Rubus leucodermis</i>	2.8 S	12.7 M	31.9 L	39.6 L	M	Medium infection; 50% of spots necrotic, others yellowed.
Golden Queen	2.9 M	9.9 L	26.8 L	35.8 L	M	Medium infection; 20% of spots necrotic, others yellowed.
Webster	1.5 S	5.4 S	33.5 M	43.5 M	S	Medium infection; 5% of spots necrotic, others yellowed.
Herbert	1.1 S	2.8 M	11.7 M	30.9 M	N	Slightly affected; spots yellowed.
Cayuga	0.1 S	2.5 S	17.7 S	29.6 S	S	Slightly affected; spots yellowed.
Ranere	4.2 S	6.5 S	14.8 S	21.7 S	M	Slightly affected; spots yellowed.
Antwerp	3.6 S	6.1 S	5.4 S	18.4 S	H	Slightly affected; spots slightly yellowed.
Seneca	0.0	0.0	2.5 S	17.7 M	S	Slightly affected; spots slightly yellowed.
Owasco	0.0	0.0	6.0 S	10.0 S	N	Slightly affected; scarcely spotted.
Cumberland	0.0	0.1 VS	2.8 VS	6.3 VS	N	Very slightly affected; scarcely spotted.
Lloyd George	0.0	0.0	3.6 VS	5.4 VS	N	Very slightly affected; scarcely spotted.

interest to observe that the Lloyd George and Antwerp, varieties of *Rubus idaeus* are much less tolerant of the disease than most of the hybrid crosses of *R. idaeus* with *R. strigosus*. The two hybrids, Chief and Latham, are quite highly resistant. According to these tests, the Cuthbert is far more susceptible than the Ranere, but, under very favorable climatic conditions near the coast in California, the latter, the standard commercial variety in central California, is much more severely damaged than the Cuthbert under the most favorable conditions for the disease in western Oregon and Washington. Thus, the data in table 1 are presented with the reservation that

the comparative susceptibility of host plants, as indicated from greenhouse tests, may not in all cases be borne out by actual field experience.

#### SUMMARY

This paper presents a continuation of our studies with the yellow rust of raspberry, which we are now convinced is caused by the European fungus, *Phragmidium rubi-idaei* (DC.) Karsten. Life history and morphological studies show it to conform closely in essential stages with those of other species of *Phragmidium* previously described by Blackman and Christman.

Precipitation and other factors, such as high humidity, simultaneously with slight lowering of temperatures, which bring about free-water on the leaf surface, are conducive to infection by the sporidia, aeciospores and urediospores.

The relative susceptibility of about 30 varieties and species of *Rubus* was tested in the greenhouse. Suffice it to mention here that, among the red raspberries, the Chief and Latham are resistant, the Lloyd George is disease-tolerant, the Antwerp, Owasco, and Seneca are fairly resistant, while the Marlboro, Cuthbert, Golden Queen, Herbert, Cayuga, and Ranere are susceptible in the order named.

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# FUNGI AND BACTERIA AS INDICATORS OF THE EFFECTS OF PETROLEUM OILS ON APPLE LEAVES<sup>1</sup>

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## INTRODUCTION

An investigation of the effects of petroleum oils and oil sprays on apple leaves made it desirable to find a convenient laboratory method for predicting toxic effects of such agents on the leaves. Hence, experiments were conducted to find an organism that would indicate such toxic effects while it was growing under oils. Fourteen species of fungi and bacteria were inoculated separately onto agar and covered with 22 petroleum oils in nearly 800 cultures and the behavior of each organism was observed and recorded.

## REVIEW OF LITERATURE

Söhngen (8) reported that species of mycobacteria oxidized benzene, petroleum, paraffin oil, and paraffin to fatty acids, CO<sub>2</sub>, and water, using the organic compounds as sources of carbon and energy. Young (12) reported that 5 species of fungi grew in undiluted oils. Young and Morris (13) used oil sprays made with many of the oils described in figure 2. Lipman and Greenberg (5) found a coccus in petroleum that decomposed petroleum. Tomkins (10) observed that fungi penetrated vaseline. Hopkins and Chibnall (3) grew *Aspergillus versicolor* on 5 synthetic paraffins including C<sub>23</sub>H<sub>48</sub> and C<sub>34</sub>H<sub>70</sub>, and on paraffin wax in mineral solutions. Stieglitz (9) mentioned that cutting oils harbored *Staphylococcus aureus*, and that paraffin oil was a favorable culture medium for such suppurative organisms.

Solubility of oxygen in paraffin oil was given a Bunsen-absorption coefficient of 0.114 at 18° C. by Washburn (11). This is calculated as meaning a concentration of 0.0185 per cent oxygen by weight in the oil; Transformer Oil and Russian Petroleum had nearly 0.025 and 0.03 per cent oxygen, respectively.

Parish (6) and Birkhaug (1) preserved delicate cultures of bacteria for 8 to 24 weeks in media under layers of liquid paraffin. Smith (7) reported that toxic oils inhibited the growth of *Fusarium cubense*. Jordan (4) stated

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that most chromogenic bacteria require oxygen for pigment production, and lack their pigments in anaerobic cultures.

#### MATERIALS AND METHODS

The oils used in this investigation are described in figure 2. They are 0 to 44 per cent sulphonatable. Nearly all of them were autoclaved in closed containers to prevent steam from entering and condensing in the oils. They were protected from contaminations.

Corn-meal agar and potato agar were used as slants, and as blocks with dimensions of  $10 \times 12 \times 2$  mm. in test tubes. Usually within 0.5 hour after inoculation and before evident growth occurred, undiluted oils were poured separately into the test tubes until the media were covered to depths of 1 to 5 cm., leaving layers of air 1 to 5 cm. thick between the oils and the cotton or cork stoppers. For comparison, some organisms were allowed to grow perceptibly before oils were poured on them. As checks for each series, organisms representing all inoculations were grown on the agar without oil. Cultures were grown at  $20^{\circ}$  to  $25^{\circ}$  C. in the laboratory. Notes were taken usually 3 and 5 days after inoculation.

#### EXPERIMENTAL RESULTS

##### Fungi Growing in Oils

*Rhizopus nigricans* Ehr. The minus strain was used in most of the tests. The plus strain was employed in some of the tests, and it reacted like the minus strain. *Rhizopus* produced many sporangia in the oils that permitted luxuriant growth (Fig. 1, A, B.). It produced sporangia and long hyphae in Oil 24 saturated with Oil Red O. Some of the hyphae in oils had irregular sheaths (fig. 1, B). *Rhizopus* grew poorly in n-decane, which was tested as a representative of the hydrocarbons in petroleum oils. It produced numerous long hyphae in oils. Its tolerance to oils resembled that of apple leaves except that *Rhizopus* was more tolerant to Oils 1, 2, 5, and 27, which are 20 to 44 per cent sulphonatable. It was the most useful fungus tested for predicting the toxic effects of oils on apple leaves.

*Mucor glomerula* (Bain.) Lend.<sup>2</sup> was isolated, in 1929, from abundant hyphae that appeared on staminal hairs of *Zebrina pendula* Schn., which were left on a microscope slide in Oil 3 under a cover glass for 8 days. These hyphae were guttulate, 1 to  $5 \mu$  in diameter, and most of them had sheaths 10 to  $15 \mu$  thick. Some branch hyphae without sheaths protruded from the sheaths. This *Mucor* grew well in many oils (Fig. 2) and sporulated in the least sulphonatable oils. It produced mycelium 10 to 20 mm. long in Oil 24 and grew well in Oil 24 saturated with Oil Red O stain, and in a mix-

<sup>2</sup> Classified by Dr. A. H. Povah, Farlow Herbarium, Cambridge, Mass.



FIG 1. A and B. *Rhizopus nigricans* (minus strain) grown for 39 days on an agar slant submerged in Oil 3. Hyphae and sporangia projected into oil 13 mm. from the agar. Note sheaths in B. A,  $\times 3$ ; B,  $\times 50$ . C. *Helminthosporium* grown for 39 days on an agar slant submerged in Oil 24.  $\times 3$ .

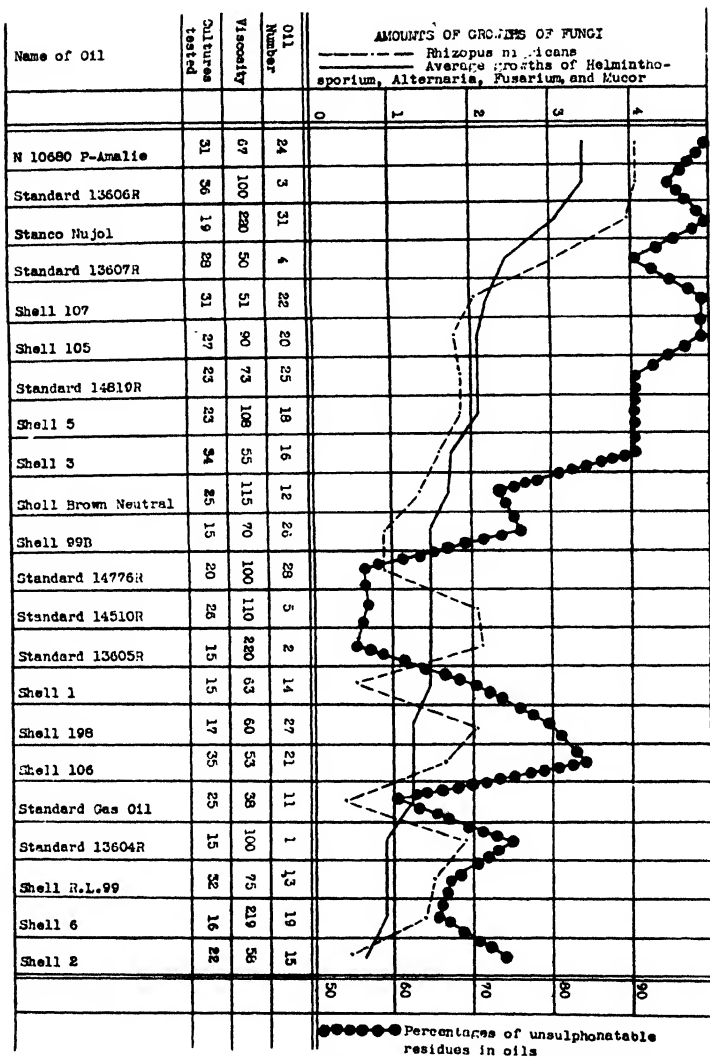


FIG. 2. Amounts of growth of 5 species of fungi in oils compared with the unsulphonatable residues of the oils. The points determining graph lines are placed at horizontal centers of the rectangles. ORDINATE (left side): Amounts of growth of these fungi in oils are indicated by numbers that signify as follows: 0 = no growth; 1 = traces of growth; 2 = moderate growth; 3 = abundant growth; and 4 = luxuriant growth with sporulation. A little emphasis was given to rapid growth within 3 days after inoculation. The standard for each fungus was its maximum growth in oils. ORDINATE (right side): The percentage of unsulphonatable residue in each oil. These percentages were determined by the oil companies, except for Oils 5, 13, 15, 16, and 21, which were determined by Green (2). ABSCISSA: The lower line gives names of oils, and symbols indicating names of oil companies; these signify as follows: *Standard* = Standard Oil Co. of California; *Shell* = Shell Oil Co.; *N* = L. Sonneborn Sons, Inc., New York; and *Stanco* = Stanco, Inc.

ture of  $\frac{3}{4}$  Oil 24 and  $\frac{1}{4}$  n-decane. *Mucor glomerula* grew best in Oils 3, 24, 4, 31, and 5, in the order listed. It did not grow in Oils 16 and 27, probably because they contained intolerable chemicals. It was nearly as useful as *Rhizopus nigricans* in predicting effects of oils on apple leaves.

*Achlya conspiciua* Coker produced long hyphae in nearly unsulphonatable oils, but it grew too sparsely to indicate clear differences in oils more than 11 per cent sulphonatable.

*Helminthosporium sativum* P., K. and B., grew well in many oils and produced abundant spores in nearly unsulphonatable oils (Figs. 1, C; 2). Hyphae lived in Oil 24 for 227 days and were nearly normal in color, size, and shape, but were very guttulate. Some hyphae bore sheaths in Oil 24. *Helminthosporium* mycelium in Oil 3 was 15 mm. long. It grew best in Oils 3, 24, 21, 5, 11, 4, 31, and 15, in the order listed, and grew very little in Oils 16, 18, 26, and 27. It grew well in n-decane, but differed from apple leaves in its tolerance for n-decane and Oils 5, 11, 15, and 21.

*Alternaria tenuis* Nees produced similar mycelium in air and oils. It grew well in n-decane and all of the oils except Oils 2, 13, 15, and 19 (Fig. 2). It produced hyphae 1 cm. long near the glass tube in a culture under Oil 25. It grew best in Oils 24, 31, 3, 4, 18, 26, and 28, in the order listed, but differed from apple leaves in its tolerance for n-decane and Oils 26 and 28.

*Fusicladium alopecuri* E. and E. grew too slowly and produced hyphae only 1 to 2 mm. long, which were too short to be valuable in indicating the toxic effects of oils. It grew best in Oils 24, 3, 31, 4, 22, 21, and 20, in the order listed, but did not grow in Oils 2, 14, and 19.

*Cladosporium* sp. grew well in Oil 3, but did not grow in Oil 5. It produced hyphae too short to be useful in further tests.

*Fusarium* sp. Culture 113 was isolated from a wilting carnation, and produced pink mycelium on corn-meal agar and hyaline mycelium in oils. It grew best in Oils 31, 3, 24, 4, and 26, in the order listed (Fig. 2), and produced hyphae 15 mm. long in Oil 3. It was a useful indicator of the toxic effects of oils on apple leaves.

*Fusarium* sp. Culture 35 was isolated from wheat, and produced white mycelium on corn-meal agar and in oils. It grew best in Oils 31, 24, 3, 12, 18, and 4, in the order listed, but did not grow in Oil 13. It grew in the other oils considered in figure 2, and produced mycelium 9 mm. long in Oil 24.

*Aspergillus* sp. grew well in many kinds of oil. It produced mycelium 10 mm. long in Oils 24 and 13, and produced some red mycelium in Oil 13.

#### GROWING BACTERIA UNDER OILS

*Chromobacterium violaceum* (Bergon.) Bergey *et al.*, showed enlargement of its inocula on agar under Oils 1, 2, 3, 4, 11, 12, 13, 16, 18, 20, 21, 22,



24, 25, and n-decane, but not under Oils 5, 6<sup>3</sup>, and 7<sup>3</sup>. The colonies did not produce their purple color noticeably under oils, but the inoculum retained much of its purple color in some cultures under all of the oils during 4 to 27 days. However, much of the purple color disappeared from the inocula under oils in some other cultures.

*Serratia marcescens* Bizio showed prominent enlargement of its inocula on agar under Oils 1, 2, 3, 4, 16, 21, 22, 24, 25, 31, and n-decane, but not on agar under the other oils listed in figure 2. The colonies did not produce their pink color noticeably under the oils, and the pink color disappeared from the inocula.

*Sarcina aurantiaca* Fluegge showed enlargement of its inocula on agar under Oils 1, 3, 5, 11, and 20, but not under Oils 4, 13, 16, and n-decane. It did not produce its yellow color under the oils, and the yellow color disappeared from the inocula under Oils 4 and 16.

*Bacillus subtilis* (Ehr.) Cohn showed enlargement of its inocula under Oils 3, 4, 16, 20, 21, 22, 24, and n-decane, but not under Oils 5, 11, 12 and 15.

*Achromobacter* sp. was isolated from agar submerged in Oil 24. The colonies were white to hyaline, long, radiating, rapidly growing strands. The bacteria were gram-negative rods 3 microns long. This species was tested under the oils concerned in figure 2, and grew on agar under all of them except Oils 1, 2, 5, and 19. It grew most under Oils 16 and 22.

The inocula of these 5 species of bacteria grew well on agar under oils less than 15 per cent sulphonatable, but grew poorly or not at all on agar under oils more than 25 per cent sulphonatable. Their colonies grew too slowly and indistinctly to be valuable in indicating the toxic effects of oils on apple leaves.

Probably too little oxygen was available in the oils for pigment production by *Chromobacterium violaceum*, *Serratia marcescens* and *Sarcina aurantiaca*. They produced their typical colors on the agar without oil.

#### Oxygen Relationships of Organism Under Oils

The organisms studied under oils were aerobes, so their growths in oils is evidence that the oils contained sufficient oxygen available to them.

Mycelium in oils usually was most abundant and long within 2 to 30 mm. below the upper surfaces of the oils, and often occurred as horizontal mats of hyphae within 2 to 5 mm. below the upper surfaces of oils in test-tubes, probably because some oxygen diffused from the air into the oils. Hyphae of *Mucor*, *Helminthosporium*, and *Alternaria* projecting away from agar surfaces in oils often were longest near the glass walls of test-tubes, and many bacterial colonies grew 1 to 5 mm. along the glass away from the agar, prob-

<sup>3</sup> Oil 6 is Standard Oil Co., Diamond Paraffin Oil. Oil 7 is Standard Oil Co., Atlantic Red Oil. Both oils had high percentages of sulphonatable residues.

ably because some oxygen adhered to the glass when the oils were poured into the tubes. Also, oxygen may enter test tubes more rapidly along the oil-glass interfaces than directly through the oils.

J. R. Green, Assistant Chemist for the Montana Agricultural Experiment Station, authorized the use of his evidence of free oxygen in the oils. He placed layers of petroleum oils 3 cm. thick over an aqueous, hyaline solution of KCNS and  $\text{FeSO}_4$ . Oils 21, 15, and 5 turned the test solution red within 1 hour, while Oils 4, 13, 3, 22, 12, 16, 20, 24, and 28 slowly turned the test solution red within 2.5 to 236 hours, in the order listed. Oil 8<sup>4</sup> very slowly turned the test solution pink within 2 weeks. This experiment gave evidence that free (dissolved) oxygen occurred in these oils, and that Oils 21, 15, and 5 apparently contained more free oxygen than the other oils.

The amounts of fungous growths in these oils was not correlated with differences in the oxygen contents of the oils as revealed in these tests by J. R. Green. The fungi secured food, water, and probably some oxygen from the agar onto which they were inoculated. Presumably they secured some oxygen from the oils. Toxic chemicals in the oils obscured any differences in fungous growths due to differences in amounts of free oxygen in the oils. *Mucor*, *Achlya*, and *Aspergillus* grew only slightly in Oil 8<sup>4</sup>, which was unfavorable to them because it was 53 per cent sulphonatable.

#### Correlation Between Growth of Fungi in Oils and Tolerance of Apple Leaves to These Oils

Each oil considered in figure 2 was painted on the Hibernial apple leaves of 3 to 25 limbs (a total of 207 limbs) in 1929, 1930, and 1931. Oils 3, 22, 24, and 31 did not kill leaf tissues within 9 to 22 days, and the bark of the attached twigs remained alive during 85 to 145 days. Oils 4, 18, 20, and 25 were more injurious to apple leaves, but they also were sufficiently innocuous for use as sprays on apple leaves. The other oils killed leaf tissues within 1 to 10 days and killed the bark of many of the attached twigs within 39 to 145 days. Oils 11, 14, 19, and 28 were the most injurious. The oils that killed parts of leaves within 1 to 10 days were too injurious for sprays on apple leaves.

Oil 16 was more injurious than its sulphonatable residue (10 per cent) indicated, probably because some of its sulphonatable chemicals were very injurious to the apple leaves and most of the fungi tested.

Tests with fungi made predictable the toxic effects of most of the oils on apple leaves because apple leaves were least injured by the oils in which the fungi grew best (Fig. 2).

<sup>4</sup> Oil 8 is Red Engine Oil, Standard Oil Co.; 53 per cent sulphonatable; viscosity of 410 sec., Saybolt.

### Survival of Fungi on Agar in Oils

Reisolations onto agar plates of fungi growing on agar under oils gave the following data on survival: (a) *Helminthosporium sativum* survived for 318 days in 2 cultures under Oil 22, and in 1 culture under Oil 5; 227 days in 2 cultures under Oil 24; 236 days under Oil 3; and 83 days under Oil 31. (b) *Rhizopus nigricans* survived 227 days under Oil 3. (c) *Mucor glomerula* survived during 94 days under Oil 12 and 43 days under Oil 13. (d) *Aspergillus* sp. survived 41 and 83 days in 2 cultures under Oil 3, 83 days under Oil 18, and 83 days under Oil 21. Fifteen of the 29 trials failed to reisolate fungi grown under oils.

The oils were poured from 320 test-tube cultures of organisms after they had been under oils during 72 days. They were in one series in which all of the oils concerned (Fig. 2) were employed. Their test tubes were stored upside down to facilitate draining the oils from the cultures. Examination 9 days later showed that new growths of the organisms had occurred in 95 of the tubes, although much oil still was on the agar. The following figures indicate the survivals of the organisms tested: 14 cultures of *Helminthosporium sativum*, 10 cultures of *Alternaria tenuis*, 4 of *Rhizopus nigricans*, 9 of *Mucor glomerula*, 6 of *Fusicladium alopecuri*, 18 of *Fusarium* sp., Culture 113, 5 of *Fusarium* sp., Culture 35, and 29 cultures of *Achromobacter* sp. Seven cultures of *Achromobacter* sp. in the toxic Oils 2, 5, 12, and 19 grew only after the oils were poured from the cultures.

Only 109 of the 349 cultures tested evidently survived the long periods of immersion in the oils described. Nearly all of the organisms that survived were growing on agar slants instead of the small, thin blocks of agar.

The oils less than 11 per cent sulphonatable were valuable preservatives. Most of the organisms grown under such oils, including the fungi shown in figure 1, A and C, made most of their growths within the first week after inoculation and did not change their appearances during the next 3 years. White oils poured on mature cultures of organisms (including *Penicillium*) on agar preserved their appearance well. Hyaline, unsulphonatable oils may become useful in differentiating physiological forms of fungi and bacteria.

### Effects of Oils on Flowing Protoplasm

Staminal hairs of *Zebrina pendula* Schn. and *Tradescantia fluminensis* Vell. on microscope slides were mounted in Oil 3 saturated with Sudan III and in Oil 24, separate lots of which were saturated with Sudan III and with Oil Red 0 (Young, 12). Protoplasm was observed to flow in the staminal hairs of *Tradescantia* 7.8 to 8.3 hours after immersion in the red-stained Oils 3 and 24 on 4 slides and 3 to 3.3 hours after immersion in these oils on 2 other

slides. Protoplasm was observed to flow in the staminal hairs of *Zebrina* for 4.25 to 6 hours after immersion in Oils 3 and 24 saturated with Sudan III on 3 slides. Later observations on these 9 slides showed no movement in the protoplasm of staminal hairs. Spheres of gas and irregular masses that probably were water appeared near the staminal-hair cells in the oils. Distortions indicating death of these cells appeared within 10 to 24 hours after their immersion in the oils. These observations on protoplasm flowing in staminal-hair cells after immersion in oils showed that these oils were only slightly toxic to the cells within 3 to 8.3 hours.

#### SUMMARY

Fungi and apple leaves usually were similar in their tolerance for petroleum oils that were less than 11 per cent sulphonatable, and in their intolerance for more sulphonatable oils. Sulphonatable chemicals differed in the ways in which they affected the different organisms.

Fungi can be used to predict the toxic effects of oils on apple leaves by using the following test: (a) inoculating agar slants in test-tubes with *Rhizopus nigricans*; (b) before evident growth occurs, submerging the agar in each tube in an autoclaved oil to be tested; (c) using Nujol or a similar unsulphonatable oil in some tubes and keeping some other tubes without oil for comparisons; and (d) incubating the cultures at 20 to 25° C. for 5 days and recording the height, sporulation, and extent of spread of the hyphae. (e) Only the oils permitting luxuriant growth of *Rhizopus* probably will be sufficiently innocuous for practical use in sprays on apple leaves.

Fungi growing under oils secured food, water, and some oxygen from the agar. Dissolved oxygen in the oils probably supplied part of the oxygen used by the long hyphae of fungi growing in oils.

The chromogenic bacteria did not produce their pigments under the oils, probably because too little oxygen was available. Most of the purple, red, and yellow colors disappeared from inocula after immersion in the oils for a week.

Protoplasm was flowing in staminal hairs of *Zebrina* and *Tradescantia* from 3 to 8.3 hours after immersion in the red-stained oils; this showed the tolerance of the staminal hairs to these oils.

Sheaths occurred on many of the hyphae of *Rhizopus* and *Mucor* in oils.

Fungi were reisolated from cultures submerged in oils for 227 to 318 days.

Hyaline, nearly unsulphonatable petroleum oils are useful in preserving the appearances of many kinds of organisms in cultures.

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# CHANGES IN PLANT-FOOD INTAKE CAUSED BY A POPULATION OF *HETERODERA MARIONI* (CORNU) GOODEY ON *ANANAS COMOSUS*<sup>1</sup>

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It is believed that nematodes cause damage to crops heavily infested by them mainly because they injure the root system and diminish its absorptive ability. Thus, Vosbury and Winston (7) state, "These nematodes (*Heterodera radiculicola*) attack the pineapple plant by penetrating and destroying its fine feeding roots, thus gradually depriving the plant of its means of taking up food and water until, when all the feeding roots are killed, the plant dies." Goodey (3) states that "*Heterodera radiculicola* is a misnomer and advocates the use of *Heterodera marioni* (Cornu) instead. Krüger (4), in discussing the effects of *Heterodera schachtii* on sugar beets, comes to the conclusion that nematodes do not damage the absorption ability of the root system but damage the beets by depriving them of their nutriment. Again, Neuwirth (6) records observations on sugar beets confirming the work of Krüger, and states that nematode-infested beets usually exhibit signs of potash deficiency. The good growth of potatoes when moderately infested with *Heterodera schachtii* led Morgan and Peters (5) to consider that "it would certainly appear that the presence of eelworm becomes unimportant when due attention is paid to the proper husbanding of the plants." The present paper describes a test instituted to determine whether or not a population of *Heterodera marioni* on pineapples reduced the absorption of nitrogen from the soil.

## EXPERIMENTAL

Sixty wooden tubs of 1220 cu. in. capacity and 11 in. depth were filled with a mixture of field soil and compost. The tubs and contents were steam-sterilized for 8 hours at 15 lbs. pressure per sq. in. Pineapple slips of the Smooth Cayenne variety were planted, one to each pot, on March 22, 1932. At the time of planting, 30 tubs were inoculated with 4 pieces of fresh English Pea root, containing galls of *Heterodera marioni*. These roots were placed about an inch below the butt of the pineapple slip. It is estimated that each tub received a charge of inoculum of 16 to 30 galls each containing 1 to 8 egg masses of about 500 individual eggs.

The pots were placed outside and they were watered at regular intervals. By June 13, 12 weeks after planting, it was very evident that the 20 plants

<sup>1</sup> Published with the approval of the Director as Technical Paper No. 34 of the Experiment Station of the Association of Hawaiian Pineapple Cannery, University of Hawaii.



FIG. 1. Comparative growth of pineapple plants with and without inoculation with *Heterodera marioni*. Two pots at left, noninoculated. Two at right, inoculated. Photographed by G. H. Godfrey on June 15.



FIG. 2. Same pots and same arrangement as in figure 1. Photographed by C. H. Spiegelberg on September 20.

inoculated with nematodes were severely retarded. Their appearance at this time is shown in figure 1.

At this time a measure of the amount of growth of each plant was attempted in the following manner: Let us refer to figure 3, showing leaves from noninoculated plants on the left and inoculated plants on the right. The distance on each from the hardened constricted line near the top to the exact tip is the length of this leaf at the time of planting. The remainder of the distance is that of growth since planting. Four leaves of each plant were selected in which the old growth was about 8 to 10 centimeters and the amount of old and new growth measured. The final results on these leaves were:

	<i>Noninoculated</i>	<i>Inoculated</i>
Total length	28.81 $\pm$ 0.44 cm.	17.11 $\pm$ 0.21 cm.
Old growth	8.80 $\pm$ 0.04 "	8.90 $\pm$ 0.16 "
New growth	20.05 $\pm$ 0.44 "	8.20 $\pm$ 0.26 "

Representative leaves of the 2 sets with lengths very near the mean of each series are shown in figure 3.

Fertilizer was applied to the lower leaf axils and to the soil adjoining the plants on June 13 at the rate of 2.2 gm. of nitrogen, 0.7 gm. of  $P_2O_5$  and 7.0 gm. of  $K_2O$  per tub. The carriers were ammonium sulphate, superphosphate, and potassium sulphate.

#### GROWTH AND DEGREE OF INFESTATION

On September 19, pictures of the same pots as shown in figure 1 were again taken. These are shown in figure 2, and, when compared with figure 1, show the growth taking place between June 13 and September 19.

On September 20, the plants were dug out and root examinations and plant weights were obtained. The procedure was similar to that of Collins and Hagan (2), except that the roots were clipped from the butt, weighed, and examined separately.

The noninoculated plants were not entirely free from galls, some slight infection having come from the planting material or tools at the time of planting. Thus in Lot A, there were five plants having galls, the numbers on each being 5, 26, 1 and 13, respectively. In Lot B, two plants had galls, the number being 2 and 4, respectively.

The number of galls on the inoculated plants was great for the age of the plant. Every plant was infected. A considerable number of the roots, however, had no galls. The majority of the large galls were concentrated on the shorter roots.



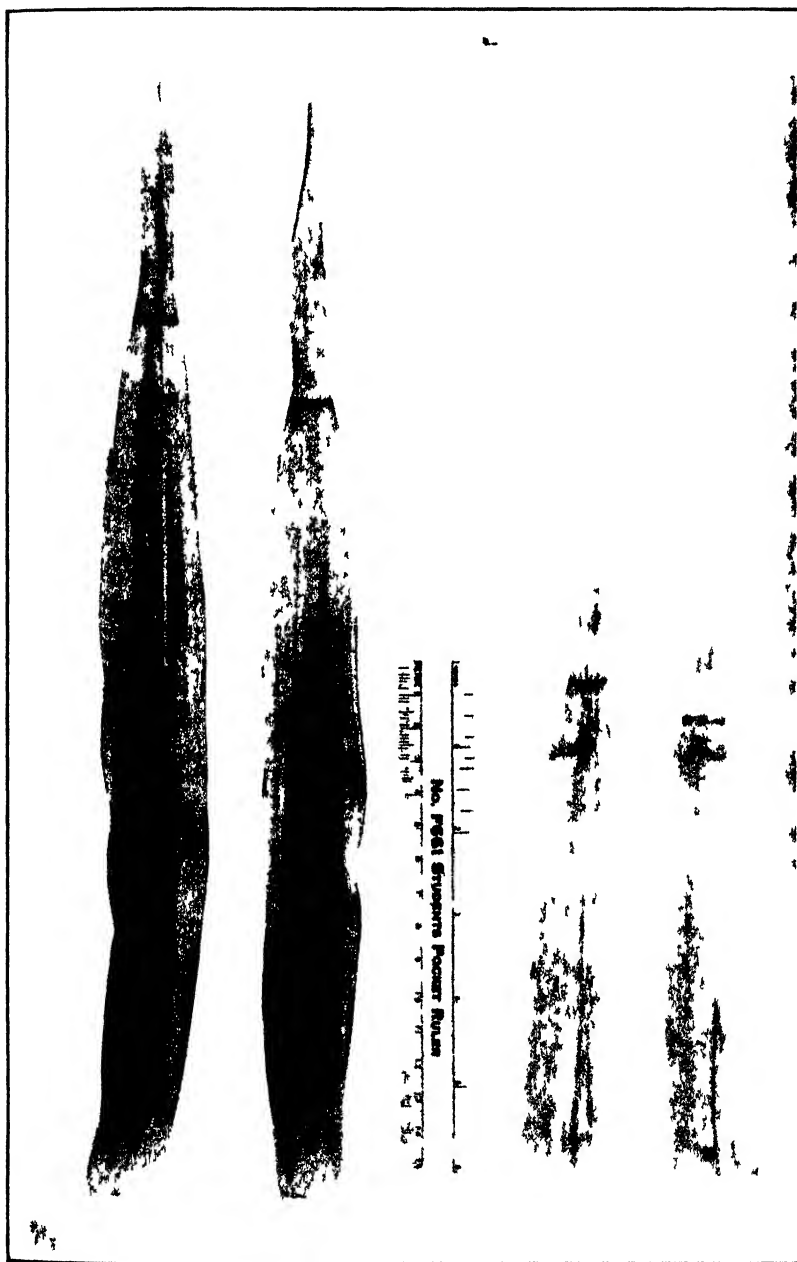


FIG 3 Comparative growth of pineapple leaves from plants with and without inoculation with *Heterodira marioni*. Leaves at left, mean of noninoculated plants. Leaves at right, mean of inoculated plants. The portion of each leaf above the transverse mark grew before planting and that below after planting.

TABLE 1.—Root number, length, weight, and number of galls per plant

Lot	Av. root wt. in grams	Av. number of roots	Av. total length cm.	Length average root cm.	Av. number galls per plant		
					Main roots	Lateral roots	Total
A <sup>a</sup>	40.49 ± 1.97	96.80 ± 2.75	1538.27 ± 70.47	15.89	1.40	1.60	3.00
B	35.95 ± 1.81	88.87 ± 2.81	1483.73 ± 64.05	16.66	0.47	.	0.47
C	32.93 ± 2.33	60.00 ± 2.65	646.53 ± 39.24	10.77	36.87	60.00	96.87 ± 11.6
D	24.62 ± 1.48	57.27 ± 2.49	681.93 ± 24.78	11.91	32.33	43.27	75.60 ± 14.7

<sup>a</sup> Treatment—A—Noninoculated, unfertilized. B—Noninoculated, fertilized. C—Inoculated, unfertilized. D—Inoculated, fertilized.

The number of galls on fertilized versus nonfertilized inoculated plants seems to indicate that fertilizer, for some reason or other, has slightly retarded the entry of nematodes into the roots. The difference in galls per plant (C—D) is  $21.27 \pm 18.7$ .

As regards root number, fertilizers have caused a slight reduction. The value of A minus B equals  $7.93 \pm 3.95$  and C minus D equals  $2.83 \pm 3.64$ ; both lack significance. Nematodes have caused a greater decrease in root numbers, the differences being:

$$A - C = 36.80 \pm 3.82$$

$$B - D = 31.60 \pm 3.75$$

Both of these decreases are very highly significant. These results are opposed to those of Collins and Hagan (2) who found no evidence that nematodes caused a decrease in root number.

In our experiment, nematodes have decreased the average total length of roots per plant with overwhelming odds.

The average percentage growth based on initial weight for the period March 22 to September 20 was: Lot A—noninoculated, nonfertilized,  $614.2 \pm 15.2$ ; lot B—noninoculated, fertilized,  $695.9 \pm 21.6$ ; lot C—inoculated, nonfertilized,  $401.0 \pm 19.5$ ; lot D—inoculated, fertilized,  $420.0 \pm 66.3$ .

A calculation to determine whether or not the difference between treatments A and C is significant gives very great odds, likewise for the difference B—D. This indicates that in this experiment nematodes did retard growth.

A slight but nonsignificant increase in growth was caused by fertilization of inoculated plants. This appears to contradict Krüger's theory that nematodes rob the plant of nutriment but do not prevent its entrance.

#### NITROGEN ABSORPTION

Six plants from each of the 4 combinations of treatments were composited. The percentages of total nitrogen were determined in these composite samples by the Gunning method modified to include the nitrogen of nitrates (1), and also in a sample of the original planting material. The nitrogen contents of the various lots were:

Lot A—noninoculated, nonfertilized,  $1.256 \pm 0.002$ ; lot B—noninoculated, fertilized,  $1.468 \pm 0.002$ ; lot C—inoculated, nonfertilized,  $1.203 \pm 0.002$ ; lot D—inoculated, fertilized,  $1.407 \pm 0.004$ .

The data above clearly indicate that fertilization has increased the nitrogen content of these plants. This effect of nitrogen fertilizers to increase the percentage of nitrogen content of pineapple plants has also been noted in a number of field experiments.

The nitrogen contents of the various lots on September 20, together with weights of the final plants, original slips, and nitrogen contents of the original slips, permit us to draw up a table showing the amount of nitrogen taken up by each plant between March 22 and September 20.

The values are as follows:

Lot A—noninoculated, nonfertilized,  $1.65 \pm 0.058$ ; lot B—noninoculated, fertilized,  $2.15 \pm 0.087$ ; lot C—inoculated, nonfertilized,  $0.89 \pm 0.032$ ; lot D—inoculated, fertilized,  $1.13 \pm 0.055$ .

We first notice that plants in A were able to obtain 1.65 grams nitrogen per plant from the soil alone. The compost in this soil is undoubtedly responsible for this large liberation of nitrogen. The difference B - A is  $0.50 \pm 0.104$ . The odds here are exceedingly great that in another experiment similarly conducted A would not equal or exceed B.

The amount of fertilizer added was 2.2 grams nitrogen per plant. If we consider that 1.65 grams were furnished by the soil, then only 0.50 gm. came from the fertilizer in B, or a utilization of 23 per cent. Heavy rains may have leached a considerable quantity of nitrogen from the soy tubs and probably prevented further absorption.

The addition of fertilizer to nematode-infested plants has increased the quantity of nitrogen assimilated per plant by  $0.24 \pm 0.066$  gm. This difference is significant with odds of 63 to 1. The data indicate that the application of fertilizer to plants infested with nematodes has significantly increased the weight and percentage of their nitrogen content, but fail to demonstrate the resultant growth (increase in dry matter).

The actual decrease in weight and percentage decrease in total nitrogen intake caused by nematodes were as follows:

Lot A - C,  $0.76 \pm 0.067$ ,  $46.1 \pm 4.4$ ; Lot B - D,  $1.02 \pm 0.103$ ,  $47.5 \pm 5.3$ .

The above values show that under the conditions of this experiment the presence of nematodes has caused a decreased nitrogen intake of 40 to 50 per cent.

#### SUMMARY

Pineapple planting material was placed in sterile soil to which, in 30 pots out of 60, an inoculum of *Heterodera marioni* had been added. Twelve weeks after planting, 15 pots of each set were fertilized and at the end of 6 months plant examinations and analyses were made. The data accumulated support the following statements as being significant, odds 30:1 or more, for this experiment:

Nematodes have decreased the average total length of roots per plant as well as the number of roots.

Nematodes have reduced the rate of growth of the plant, as judged by dry-matter contents.

Fertilizers seem to have increased the rate of growth of noninfested plants, odds 25:1 but not that of the infested plants.

The presence of nematodes retarded the absorption of nitrogen by 40 to 50 per cent.

Application of fertilizer has markedly increased the percentage of nitrogen in the plant but has resulted in increased growth only in the case of non-infested plants.

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# AN AUTOMATIC MOISTURE-RECORDING DEVICE

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The duration of periods of wetting by rain or dew is often an important factor governing infection under field conditions. In experimental work it is desirable to know the length of these periods and when they occur, but it is obviously impractical to make observations frequently enough to determine the exact lengths of probable infection periods throughout an entire season. It is, therefore, desirable to have an automatic device to record the length of time the leaves remain wet.

Many organisms require the continuous presence of a film of water for several hours before they cause infection. In developing the device described below, it was, therefore, decided that it should be operated by the film of moisture on the surface of the leaf. Since this film could not provide the energy needed to operate a recording device, it was decided to use the film to control an electrical circuit from which the necessary energy could be obtained. However, if an electric current were passed through the water film, electrolysis would result, and the products thus formed might injure the leaf. It was, therefore, evident that the recorder must be operated by voltage and not by current. This requirement was met by using a vacuum tube.

A semipictorial diagram of the electrical circuit appears in figure 1, A. A type '45 radio tube is supplied with voltage from a simple "B eliminator," which uses a type '80 rectifier. Sufficient filtration of the direct current is obtained with a single 8-microfarad condenser, C. A 10,000-ohm resistor,  $R_1$ , in the negative plate-return lead furnishes a grid bias voltage for the '45 tube. The negative end of this resistor is connected to earth. The grid lead of the '45 tube is connected to a small metal plate, M, which is separated from the leaf of a plant by means of a thin insulator, I. When the leaf becomes wet the water film bridges across this insulator, making electrical contact between the metal plate and the leaf. Since the leaf is grounded through the plant, the negative bias voltage developed across  $R_1$  is applied to the grid of the tube, causing a large decrease in the plate current. When the leaf dries, the grid circuit is opened, and the plate current increases. In addition to providing the biasing voltage for the '45 tube, the resistor  $R_1$  also prevents the current in the plate circuit from increasing to a point where it might damage the tube or other parts of the apparatus when the grid voltage is removed from the tube. The resistor  $R_2$  is a "bleeder" across B + and ground.



may be interpreted in terms of wetness or dryness of the leaf, is made on the drum.

In order to obtain sufficient power to operate the recording pen, it is necessary to use a tube with a fairly large plate current. The '45 proved most satisfactory, although a '71-A has also been tried, with about the same degree of success. The electromagnet used is actually a Western Electric type R-1314 telephone relay, with the relay contacts bent up out of the way. If one wished to operate some device other than a recording pen, it would be quite feasible to use the contacts of the relay for opening and closing appropriate electrical circuits. In this way lights, bells, motors, or other electrical equipment could be controlled. For such purposes, the moisture apparatus could be operated from batteries, using either a 201-A or 199 tube, provided that one employed a relay that was sufficiently sensitive to operate on the smaller plate currents of these tubes.

The pen is attached to the armature of the relay and the latter mounted in the case of a recording thermograph, which originally contains a revolving drum operated by clockwork, and another pen that records the temperature on this drum. The moisture-recording pen is placed near the top of the drum in order not to interfere with the temperature-recording pen. Thus both records are made on the same chart. If a recording thermograph were not available, it should be possible for anyone with a little mechanical ingenuity to devise a suitable substitute for the moving drum. In many cases, however, the thermograph is part of the field equipment and represents no extra expenditure. As mentioned above, the relay contacts are bent up out of the way. This is done to allow the armature to move more freely and to obtain a greater movement of the recording pen. A small coil spring is added to pull the armature away from the magnet when the plate current decreases. Such a spring can be made from fine piano wire, or a small weight might be fastened to the armature to obtain the same effect.

All of the parts except the relay and the metal plate, M, are mounted on a metal chassis. This may be made from a sheet of heavy galvanized iron by bending its edges down at right angles to provide about  $1\frac{1}{2}$  inches of space beneath for the wiring and for mounting the tube sockets and resistors. There should be two  $1\frac{1}{8}$  inch holes in the chassis for mounting the tube sockets and such other holes as are required for the wiring and for mounting the transformer, condenser, and resistors. The transformer and condenser should be mounted on top of the chassis and should be located so that the wiring will be as simple and direct as possible. All electrical connections should be well soldered, using rosin flux. Acid flux must *never* be used for electrical work, since electrolytic effects are set up when current flows and the joint is quickly destroyed.



The metal plate, M, may be of thin copper, or brass or heavy tinfoil. It is soldered to a wire that connects it to the grid of the '45 tube. This wire should be enclosed in either glass or rubber tubing, sealed moisture-tight at each end with paraffin. It is *absolutely imperative* that this wire be kept dry and thoroughly insulated from the earth (and metal chassis), as even a high resistance contact between grid and earth is sufficient to cause a decrease in plate current and, therefore, to operate the recording pen.

The insulator, I, may be a thin piece of glass, such as is used for a microscope cover slip, or a small piece of mica, rubber, or other good insulator of sufficient thinness that a film of water can creep over it and make electrical contact between metal plate and leaf. The thickness and nature of the insulator determine to some extent the sensitiveness of the device. Thin glass insulators may even cause the device to operate in a very humid atmosphere, apparently because the glass condenses a film of moisture on its surface. A fairly thick rubber insulator ( $\frac{1}{8}$  to  $\frac{1}{4}$  inch) will usually prove more satisfactory. The insulator may be fastened to the metal plate by means of shellac, but care must be taken that the top of the metal plate be not insulated with a coating of shellac. In very moist localities, such as those near the seacoast, the device may prove too sensitive for satisfactory operation unless special precautions are taken to protect all parts against the extreme humidity.

Figure 1, B, shows the method by which the connection between leaf and grid is made in the field. One end of a strip of wood, B, about  $\frac{3}{4}$  inch wide is attached to some suitable support so that the free end is placed where a leaf may be clamped to it without injury to the leaf. It is probably best to clamp the leaf in a horizontal position. The wire, W, with the metal plate, M, attached, is passed through a piece of glass tubing, G', and the metal plate brought into contact with the end of the glass. The metal plate is then sealed to the glass tubing by means of paraffin, care being taken not to insulate the entire upper surface of the metal with paraffin. This tube, with the metal plate and insulator attached, is held in position by a wooden clamp, C', so that the insulator rests upon the leaf. A rubber band, R', holds it firmly against the leaf and effectively clamps the leaf between the insulator and the wood strip, B.

Tube G' is connected to a second piece of glass tubing, G'', by means of a piece of rubber tubing, T'. The entire outside surface of the glass and rubber tubing is coated with paraffin and the end of tube G'' is sealed with the same material. Tube G'' should carry the wire through the case that houses the electrical apparatus. The wire should be connected directly to the grid contact of the tube socket, but care must be taken not to allow the wire to touch any of the other metal parts of the apparatus.

It would be satisfactory to pass the wire through one long piece of glass tubing, sealing the ends with paraffin, but such an arrangement is fragile and lacks flexibility. The rubber tubing furnishes this flexibility, while the glass tubing at either end furnishes rigidity for clamping.

For use in the field, the apparatus is housed in a small weather-tight house, D, patterned after the government weather stations. An upper shelf holds the metal chassis, while a lower shelf, having ventilating shutters at the sides, accommodates the thermograph and relay. The house is located close to a plant of the species being used for experiment and the connection made to a leaf. The metal chassis is connected to earth by means of a wire, thus completing the circuit through the plant. The location must also be close to an available source of 110-volt alternating current.

The parts required for construction of the moisture-recording device are as follows:

Two UX-type (four-prong) "wafer" tube sockets; 1 type '45 radio tube; 1 type '80 radio tube; 1 resistor, 10,000 ohm, 25 watt ( $R_1$ ); 1 resistor, 25,000 ohm, 25 watt ( $R_2$ ); 1 a.c. switch (Sw); 1 dry electrolytic condenser, 8 microfarad, 450 volt (C); 1 power transformer (T) with the following windings: (a) 110 volt primary; (b)  $2\frac{1}{2}$  volt,  $1\frac{1}{2}$  ampere secondary, center tapped, for '45 tube; (c) 5 volt, 2 ampere secondary, center tapped, for '80 tube; (d) high voltage secondary, center tapped, supplying 250 to 300 volts each side of center. One relay, Western Electric type R-1314; 1 recording thermograph, or substitute for moving drum; 1 metal chassis; 25 feet of "push-back" insulated wire; rosin-core solder; bolts and nuts; glass and rubber tubing, etc.

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# ONION-BULB DECAY CAUSED BY *ASPERGILLUS ALLIACEUS*<sup>1</sup>

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(Accepted for publication May 29, 1933)

## INTRODUCTION

*Aspergillus alliaceus* Thom and Church is a yellow-spore, sclerotium-forming species, that has been intercepted twice on garlic imported into this country from Italy. In the first case it was found in a shipment arriving in New York City in 1919, and in the second instance in a shipment that arrived at the port of Charleston, South Carolina, in 1929. The fungus was described by Thom and Church<sup>2</sup> on the basis of a culture isolated from the first collection. They reported another strain, similar in morphology, which was obtained from a dead blister beetle. As already pointed out,<sup>3</sup> this fungus was shown to be an aggressive decay-producing parasite when inoculated through wounds into mature bulbs of onions and garlic. It has never been reported in nature upon onion, but since it has been introduced into this country with garlic shipments it became important to study its potentialities as the cause of an onion disease.

## THE CAUSAL ORGANISM

Thom and Church<sup>2</sup> give a detailed description of the organism, including its cultural characters on Czapek's solution agar. On this substrate white aerial mycelium spreads rapidly over the surface, becoming less pronounced as the sclerotia are produced. Conidiophores are produced bearing heads, yellow at first, but becoming brown with age. The sclerotia are white at first, turning brown and finally black. The darkening begins at the end of the sclerotium in contact with the substrate. The upper end remains white for a time, and at this stage it usually bears a colorless, glistening drop of liquid that disappears as the entire sclerotium becomes black. Growth on potato-dextrose agar is quite similar, except that the aerial mycelium in the early stages is more profuse.

The growth on potato-dextrose agar in Petri dishes was studied over a range of constant temperatures. Growth was meager at 15° C. and below. The rate of radial expansion increased directly with the temperature up to an optimum at about 32° C. Growth was greatly retarded at 40° C. and

<sup>1</sup> Cooperative investigations between Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and Department of Plant Pathology, University of Wisconsin.

<sup>2</sup> Thom, C., and M. B. Church. *The Aspergilli*. 272 p. Williams & Wilkins Co., Baltimore. 1926.

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the maximum temperature is somewhat above that point. The South Carolina isolate produced conidia more abundantly at most temperatures, while the New York isolate produced sclerotia in greater numbers (Fig. 1). Sclerotia developed most promptly at 32°, and progressively less rapidly at higher and lower temperatures, except at 40°, where none occurred. At 20° and below they were somewhat larger than at higher temperatures. Conidiophores were produced most abundantly at 40°, where they were closely crowded together. At 32° they were numerous but more widely scattered. At lower temperatures they were relatively sparse.

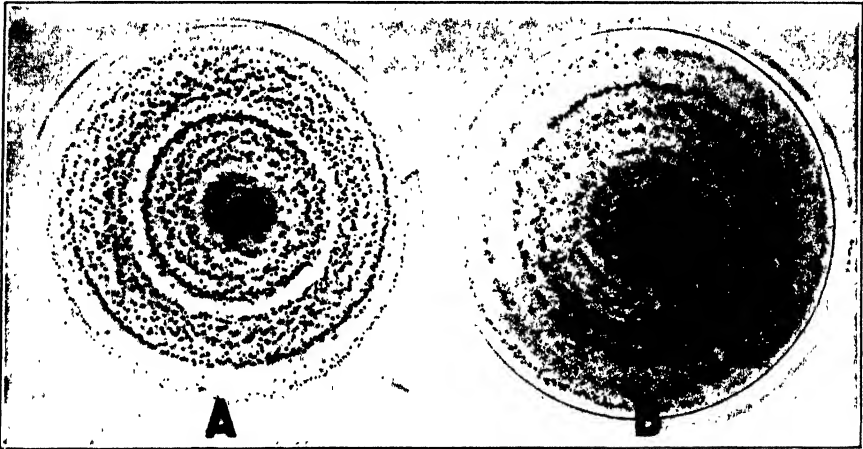


FIG. 1. Petri-dish cultures of *Aspergillus alliaceus* on potato-dextrose agar, 9 days after inoculation at 32° C. A. New York isolate. B. South Carolina isolate. Note greater conidial production in B.

#### PATHOGENICITY

The pathogenicity of the fungus on mature onion bulbs and on garlic has been demonstrated repeatedly by inoculating them through wounds and placing them in moist chambers or in glassine bags. The first sign of the disease is a slight swelling and darkening of the tissue around the point of inoculation. The bulb tissue becomes watersoaked and softens and individual cells are affected slightly in advance of the hyphae. A distinct line of demarcation occurs between the advancing lesion and the unaffected healthy tissue. The older decayed tissue shrinks and becomes brownish. At favorable temperatures a dense, white mycelium mat forms between the bulb scales. Sclerotia are formed, first around the point of inoculation and later throughout the decayed tissue. They are white at first, turning black as they mature. Conidiophores bearing conidia, yellow *en masse*, are borne in abundance on the surface of the decaying bulb at high relative

humidity. Premature sprouting of affected bulbs is often stimulated. The decaying tissue desiccates readily, assuming a dry, spongy texture.

Inoculated onion bulbs were placed in glassine bags and incubated at constant temperatures varying from 8° to 44° C. After 21 days the bulbs at 36° C. were completely rotted and progress of the disease was nearly as rapid at 28° and 32°. At 20°, 24°, and 40° there was very slight decay, while at 16° C. none developed during a period of three months.

The possibility of the fungus becoming a parasite of growing onion plants was investigated. Cultures on a mixture of sterilized corn-meal and sand were allowed to grow until sclerotia had formed in abundance. They were mixed thoroughly with greenhouse compost soil, which was placed in 5 containers and inserted in soil-temperature-control tanks, one each at 16°, 19°, 22°, 26°, and 30° C. Four White Portugal onion sets were planted in each container and allowed to grow for 83 days. The moisture content of the soil was held at approximately 60 per cent of the water-holding capacity for 34 days and at 80 per cent for the remainder of the period. No signs of disease development occurred on any of the plants at any temperature during the course of the experiment. Two tanks were set up at 28° C. containing 12 containers of inoculated soil and 4 containers of noninoculated soil. Ten White Portugal onion sets were planted in each container. In one-third of the containers the sets were each wounded with a needle before planting. Fifteen days later the plants in another third of the containers of inoculated soil were wounded near the bases of the scales and mycelium from a pure culture injected into the wound. When the plants were all pulled and examined 53 days after the experiment was started, no signs of disease were noted in any of them. It is apparent that the fungus does not attack growing onion plants.

#### DISCUSSION

The organism has been found twice causing a decay of imported garlic bulbs. It also is pathogenic on onion bulbs, although it has not been reported upon the latter in nature. Compared with other decay-producing parasites of onion bulbs, *Aspergillus alliaceus* is a high-temperature organism, its optimum activity being within the range of 28° to 36° C. This fact probably limits its potential danger as the cause of a serious disease in this country, since it can be effectively checked at storage temperatures below 20° C. It does not attack the growing onion plant and appears to be confined in its parasitic activity to the mature bulbs.

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# VARIETY TESTS IN THE DIFFERENTIATION OF TWO COTTON WILTS <sup>1</sup>

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(Accepted for publication May 9, 1933)

Wilt caused by *Fusarium vasinfectum* Atk. is the most destructive disease of the cotton plant in most cotton-growing regions where the even more destructive *Phymatotrichum* root rot is not present. In Texas, wilt occurs more generally in the sandy-loam soils in the eastern part of the State. It has been shown previously <sup>2</sup> that these soils in which wilt occurs are generally somewhat acid in reaction. *Phymatotrichum* root rot, on the contrary, is most prevalent in heavy, neutral to basic soils. It was, therefore, a matter of special interest when, in 1927 and 1928, a wilt was found attacking cotton near Waxahachie, Ellis County, Texas, in alkaline, heavy clay soils in which *Phymatotrichum* root rot also was prevalent.<sup>3</sup> The symptoms of this wilt differed somewhat from those of the typical *Fusarium vasinfectum* wilt. With *Fusarium* wilt the stem of the cotton plant was discolored mostly in the outer woody tissue, while with the new wilt the discolored area was confined mainly to the interior cylinder of both roots and stems. *F. vasinfectum* was rarely among the fungi isolated from the diseased plants from Waxahachie; however, none of the various other fungi isolated from the plants have yet been proved the cause of the disease. It, therefore, still remained in some doubt whether the new wilt (which for convenience is designated temporarily as Waxahachie wilt) might not be, perhaps, the well-known *Fusarium* wilt adapted to calcareous soils.

In 1929 a field heavily infested with *Fusarium* wilt was located near Tabor, Brazos County, and this afforded opportunity to test some of the standard wilt-resistant varieties for resistance to *Fusarium* wilt as it occurs in Texas. At the same time, parallel plantings of the varieties were made at Waxahachie, to find whether the new wilt resembled *Fusarium* wilt in its effect on different varieties of cotton. Part of each lot of seed was planted in replicated rows in the field near Tabor, and the remainder of each lot in replicated rows in the field at Waxahachie. Total numbers of plants ranged from 150 to 200 plants of each variety in the 1929 test at Waxahachie to 500

<sup>1</sup> Published with the approval of the Director as Technical Contribution No. 265 of the Texas Agricultural Experiment Station.

<sup>2</sup> Taubenhause, J. J., Walter N. Ezekiel, and D. T. Killough. Relation of cotton root rot and *Fusarium* wilt to the acidity and alkalinity of the soil. Texas Agr. Exp. Sta. Bul. 389. 1928.

<sup>3</sup> Taubenhause, J. J., W. N. Ezekiel, and H. E. Rea. A new cotton wilt. Phytopath. 19: 171-173. 1929.

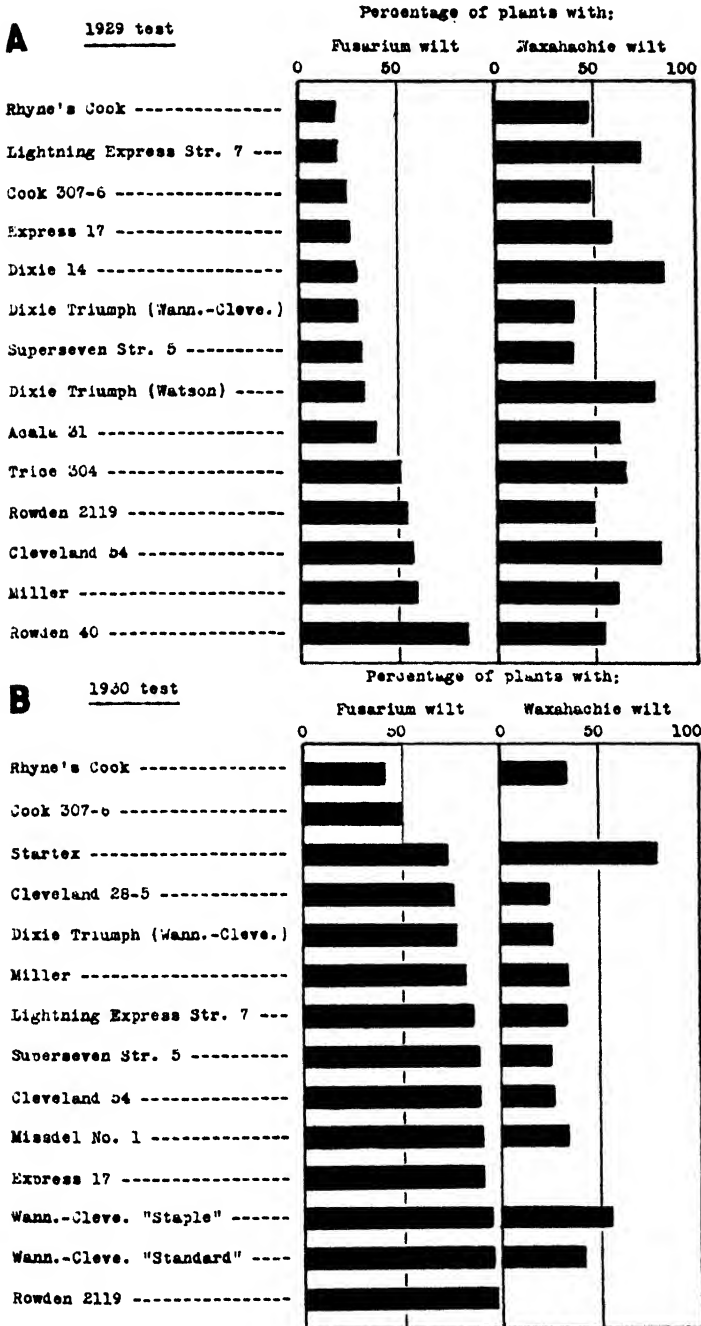


FIG. 1. Percentages of cotton plants killed or with characteristic internal discoloration. A. 1929. B. 1930.

to 700 plants of each variety in the 1930 test at Tabor. At the end of the season each plant was pulled and the stem and root cut open and examined. It was possible thus to determine not only the percentages of plants killed, but also the percentages of living plants that showed internal symptoms of injury.

The results obtained in 1929 and 1930 are shown in figure 1. In these graphs, the total percentages of plants attacked by *Fusarium* wilt in the tests in Brazos County are shown on the left, and those of plants attacked by Waxahachie wilt in the tests in Ellis County on the right. Quite obviously, the varieties did not react in a similar manner to these 2 diseases. For 1929, the 5 varieties most resistant to *Fusarium vasinfectum* wilt were instead 3rd, 11th, 5th, 7th, and 14th in the order of resistance to Waxahachie wilt. The Watson and Wannamaker-Cleveland selections of Dixie Triumph cotton were almost equally resistant to *Fusarium* wilt, while they reacted quite differently to Waxahachie wilt. Taken as a whole, there was no significant correlation in the reaction of the varieties to the 2 diseases.

Similar results were obtained in 1930 (Fig. 1, B). Conditions were very favorable for the *Fusarium* wilt in Brazos County and heavy losses occurred in the test plots. As in the preceding year, the variety Rhyne's Cook showed the least wilt infection, and its mean yield and that of Cook 307-6, from which Rhyne's Cook was originally selected, were more than double the yields obtained from the other varieties. Yet, Rhyne's Cook was 7th in resistance to Waxahachie wilt; and Startex was lowest in resistance to this disease, although it had been 3rd in resistance to *Fusarium* wilt. There was again no significant correlation between the reactions of the varieties to the 2 diseases.

In 1931 and 1932, wilt-resistant varieties were tested against *Fusarium vasinfectum* wilt only in plots at College Station. These additional results with *Fusarium* wilt, which were also in accord with results of cotton-variety tests in other States, yielded further evidence of the reasonably stable reaction of varieties to this particular disease as shown by percentages of infected plants. Thus Rhyne's Cook led in freedom from infection for 2 years, and was second best for the other 2 years. Cook 307-6 ranked 3rd, 2nd, and 1st, respectively, during the 3 years it was included in the tests. Among the less resistant varieties, Rowden 2119 ranked 11th, 14th, 18th, and 11th again during the 4 years; and Cleveland 54 ranked 12th, 10th, 12th, and 16th. Kasch and Gorham's Lonestar were lowest in wilt resistance in both years that they were tested. Detailed results of these variety tests will be given in a separate paper.

#### SUMMARY

Cotton varieties have been tested in Brazos County, Texas, for resistance to *Fusarium vasinfectum* wilt, and in Ellis County for resistance to an appar-



ently different wilt found there and designated temporarily as Waxahachie wilt. Varieties resistant to *Fusarium vasinfectum* wilt were generally consistently resistant, while susceptible varieties were fairly consistently susceptible in the various years. Varieties resistant to this disease were not, however, necessarily resistant to the Waxahachie wilt, nor was susceptibility to the one disease correlated significantly with susceptibility to the other. It therefore appears reasonable to conclude that the differences between the reactions of cotton varieties to the Waxahachie wilt and those to known *Fusarium vasinfectum* wilt constitute an additional distinction between the two diseases.

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# QUANTITATIVE DETERMINATION OF SULPHUR ON LEAVES BY TITRATION

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Numerous quantitative determinations of the amount of sulphur adhering to the foliage of trees treated with sulphur fungicides have been made by the writer during the course of 5 years' experimental work with the dusting and spraying of apples. A gravimetric method of analysis, combining the more desirable features of methods used by Fitch<sup>1</sup> and Streeter<sup>2</sup>, was used for most of this work. Because this method involves a long procedure and requires equipment and facilities difficult to obtain in a field laboratory, a new method<sup>3</sup> has been developed, the basis for which was suggested to the writer by R. Winters, of the Department of Plant Pathology, Cornell University.

This new method is based upon the volumetric determination of the amount of sodium hydroxide used when the sulphur is oxidized to sodium sulphate. The sulphur is first heated with a known amount of a strong solution of sodium hydroxide until all of the sulphur has reacted to form soluble sulphides or thiosulphates. These compounds are then oxidized to sodium sulphate by the action of hydrogen peroxide. When the reaction is complete the remaining sodium hydroxide is determined by titration with a standard solution of hydrochloric acid. The amount of sulphur can then be calculated from the amount of sodium hydroxide used in the reaction.

The analytical procedure may be outlined briefly as follows:

1. Collect representative samples of 150 leaves each, placing them directly into glass beakers. If possible, each sample should consist of equal numbers of leaves taken from 3 or more trees. Duplicate samples may be taken in order to check the accuracy of the procedure.
2. Make a separate determination for each sample, as follows:
  - a. Pour about 300 cc. carbon tetrachloride into a large beaker. Immerse about 40 leaves in the tetrachloride and let stand for 10 minutes. Turn the leaves over with a glass stirring rod and let stand for another 10

<sup>1</sup> Fitch, H. W. Revised method for the quantitative determination of sulfur fungicides on foliage. *Phytopath.* 16: 427-428. 1926.

<sup>2</sup> Thatcher, R. W., and L. R. Streeter. The adherence to foliage of sulfur in fungicidal dusts and sprays. N. Y. (Geneva) State Agr. Expt. Sta. Tech. Bul. 116. 1925.

<sup>3</sup> After this work was completed the writer learned that Fr. Kühl, working at Copenhagen, used the same method for analyzing weighed samples of sulphur. This was published in the following article:

Kühl, Fr. Ein Schnellverfahren zur Gehaltsbestimmung von Schwefel. *Ztschr. Analyt. Chem.* 65: 185-186. 1924.

minutes. Remove the leaves a few at a time, pulling them out by the stems, and rinse in a second beaker containing about 250 cc. of carbon tetrachloride. Carefully shake off all droplets of the solvent and discard the leaves. Immerse successive 40-leaf portions of the sample in the first beaker and repeat the washing until all of the sample has been treated.

- b. Filter the contents of both beakers through a thin tuft of absorbent cotton and place the filtrate in a 500-cc. florentine flask. Rinse all beakers and the filter with clean carbon tetrachloride and add the rinsings to the flask.
- c. Place the flask on a hot plate and distill until the volume of the sample has been reduced to about 10 cc. Save the distilled solvent for future use.
- d. Heat the sample on a steam bath until *all* of the solvent has been removed.
- e. Add 5 cc. of distilled water and make neutral to methyl red indicator, using approximately 0.1 normal solutions of either hydrochloric acid or sodium hydroxide.
- f. Using a pipette, add 20 cc. or more<sup>4</sup> of a 2.0 normal solution of sodium hydroxide, close the flask with a "protection tube"<sup>5</sup> (Fig. 1), and heat on the steam bath until there is no free sulphur remaining in the flask.
- g. Allow the sample to cool to room temperature and then slowly add a 30 per cent solution of hydrogen peroxide, using 5 cc. for each 20 cc.

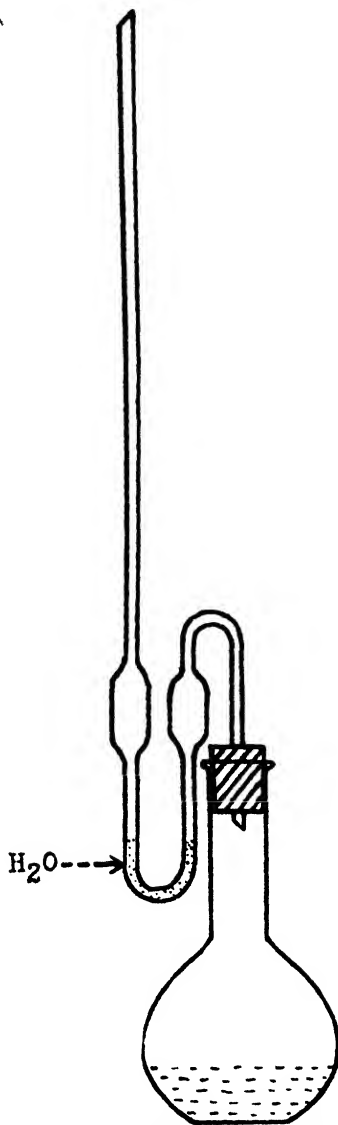


FIG. 1.—A satisfactory protection tube. Its use prevents any loss of solution from the flask.  $\times \frac{1}{4}$ .

<sup>4</sup> The exact quantity of sodium hydroxide to be added to the flask will depend upon the amount of sulphur expected in the sample, each 0.5 gram of sulphur requiring about 20 cc. of the sodium hydroxide solution. If free sulphur remains in the flask after 5 to

of sodium hydroxide. Heat on the steam bath for 3 to 5 hours, cool, and add a second portion of hydrogen peroxide. Heat again for 1 hour and then allow to cool.

- h. Titrate the remaining sodium hydroxide against a standardized 1.0 normal solution of hydrochloric acid, using methyl red indicator.
3. Run a blank determination with each set of samples, using the same amount of sodium hydroxide as was used in the samples. Titrate this blank to determine the exact amount of sodium hydroxide used.
4. Calculate the amount of sulphur present in each sample, as follows:
  - a. cc. HCl used to titrate blank - cc. HCl used to titrate sample = cc. HCl equivalent to NaOH used to form  $\text{Na}_2\text{SO}_4$
  - b. cc. HCl equivalent to NaOH used to form  $\text{Na}_2\text{SO}_4 \times$  "factor" = grams of sulphur
  - c. "factor" = normality of standard HCl solution  $\times$  0.016

This method of analysis was tested extensively during the summer of 1932. Samples analyzed by this method were later analyzed gravimetrically in order to compare the 2 methods. Data obtained from 48 comparisons show that when the amount of sulphur in the sample is greater than 0.050 gram the variation in the amount analyzed by the 2 methods is considerably less than the variation found when duplicate leaf samples are analyzed by either method. The amount found by titration is usually slightly greater than that found gravimetrically, averaging 2.9 per cent more. When the amount of sulphur is less than 0.050 gram a higher variation is found between the analyses obtained with the 2 methods. This appears to be due to inaccuracy in the titration method.

Preliminary tests were made in an effort to increase the accuracy of the method with samples containing small amounts of sulphur. A series of duplicate samples, each containing 0.003 gram of sulphur, was analyzed, using 5 different strengths of sodium hydroxide and 4 of hydrochloric acid. The results obtained indicate that the accuracy of the method can be increased by the use of more dilute solutions. The use of 0.5 normal hydroxide and 0.25 normal acid reduced the error to about 10 per cent. The accuracy of the method was still further increased by the use of 0.125 normal

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8 hours of heating more sodium hydroxide should be added. Using 150-leaf samples, the writer has found amounts of sulphur varying from slightly over 2.0 grams to as little as 0.003 gram.

<sup>5</sup> The "protection tube" is used to prevent the oxygen that is evolved from carrying a fine mist of solution out of the flask. Protection tubes may be made from large-bore glass tubing, bending it so that gases can enter or leave the flask only after bubbling through a column of distilled water in the tube. The contents of the tube should be washed into the flask just before making the titration. Figure 1 shows a more satisfactory type of protection tube.

alkali and 0.10 normal acid, but, with such a weak alkali, the reaction with sulphur proceeds very slowly, thus greatly increasing the time of heating.

It is not claimed that this method is sufficiently accurate for delicate chemical analyses. However, the method seems to be entirely satisfactory for the purpose for which it was developed. It has the advantage of requiring much less time and equipment than does the gravimetric method and should prove useful wherever approximate quantitative analyses of free sulphur are desired, provided the material to be analyzed does not contain impurities that will react with the sulphur or with sodium hydroxide.

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## PHYTOPATHOLOGICAL NOTES

*A New Species of Graphium Causing Lumber Stain.*—A red-headed species of *Graphium* has been found frequently on freshly cut sapwood in forests and lumber yards. It has thus far occurred always associated with other blue-stain fungi, *Ceratostomella pilifera* (Fr.) Wint., *C. pluriannulata* Hedg., and *Graphium rigidum* (Pers.) Sacc.

This fungus has been collected in Ohio, Arkansas, Mississippi, and South Carolina. In forests it occurred on freshly felled *Populus deltoides* Marshall, *Quercus alba* L. and *Q. lyrata* Walter and in lumber yards on sap wood of *Liquidambar styraciflua* L. and on a *Pinus* log.

The synnemata of the fungus are noticeable because of the carmine<sup>1</sup> color of the droplets on the tips of the stalks. This carmine-colored mucous matrix, which holds the primary conidia, is soluble in water. It sometimes flows down the stalks and stains the surrounding surface of the wood making it look as if splashed with blood. The external red stain is transitory and of minor importance, compared to the blue stain caused by the vegetative hyphae growing in the wood cells.

To test the staining ability of this *Graphium*, sticks of freshly cut sap wood of sweet gum (*Liquidambar styraciflua*) were inoculated with a pure strain of the fungus, which had been isolated from sap gum. The wood was stained gray or grayish black when damp. The fungus fruited vigorously. Pine, *Pinus echinata* Mill. and *P. taeda* L., was inoculated with strains isolated from gum. The wood was stained a light gray. The fungus fruited readily. The stain it caused is not so dark as that caused by the common yard stain fungi, *Ceratostomella pilifera* and *Graphium rigidum*, and it is not so vigorous in culture. The writer published a preliminary description of this fungus in 1911.<sup>2</sup>

### *Graphium rubrum* sp. nov.

#### DIAGNOSIS

Stipitibus fertilibus 780 $\mu$  altis, 45 $\mu$  crassis, fuscis nigrescentibus. Capitibus ex hyphis hyalinis compositis conidia prima acrogena gerentibus. Conidiis in carmine coloratos mucosos, globosos, 200 $\mu$  crassos cadentibus, oblongis, hyalinis, 2 $\times$ 4 $\mu$ . Mycelio septato, 1–4 $\mu$ , nigrescente. Conidiis secundariis hyalinis, claviformibus ex conidiophoris erectis nascentibus, acrogenis elatis et capitulatis, forma diversis.

<sup>1</sup> Ridgway, Robert. Color Standards and Nomenclature. Washington, D. C. 1912.

<sup>2</sup> Über die Einwirkung des Säure und Alkaligehaltes des Nährbodens auf das Wachstum der holzersetzenden und holzverfärbenden Pilze, mit einer Erörterung über die systematischen Beziehungen zwischen *Ceratostomella* und *Graphium*. Naturwissenschaftliche Zeitschrift für Forst und Landwirtschaft 9: 429–467. 1911.

Hab. in ligno sapido *Quercus albae*, *Populi deltoidis*, *Quercus lyratae*, *Liquidambaris styracifluae* et *Pini* sp. in America boreale.

***Graphium rubrum* sp. nov.**

TECHNICAL DESCRIPTION

Synnemata dark brown or black, expanded at base, 480–2000 $\mu$ , average 780 $\mu$ , in height, and 9–86 $\mu$ , average 45 $\mu$ , in diameter (Fig. 1); head com-

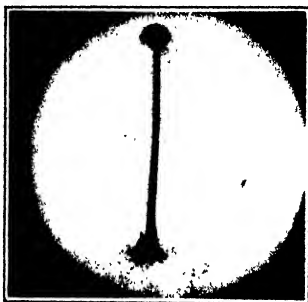


FIG. 1. Synnema of *Graphium rubrum* without the carmine mucous globule covering the head.  $\times 75$ .

posed of hyaline branched hyphae bearing primary conidia terminally by abstriction; conidia drop into a carmine-colored mucous that forms a globule covering the surface of the head; globule 18–425 $\mu$ , average 200 $\mu$ , in diameter; young synnemata frequently grow from the heads of old ones; there is occasional proliferation of the synnemata. Primary conidia oblong, hyaline, average  $2 \times 4\mu$ ; under favorable conditions conidia germinate producing mycelium; under unfavorable, they bud like yeast. Hyphae septate, 1–4 $\mu$ , in diameter; the young culture hyaline, changing with age to gray, slate, and slate black. Slow-growing mycelium forms dense, white, glistening mats of hyphae and sometimes sterile, clavariiform fascicles. Secondary conidia club-shape, hyaline, develop first on hyphae, later on simple erect conidiophores, borne at the tips and collecting in clusters; vary in size and shape  $3.2 \times 8.5\mu$  to  $2 \times 14\mu$ , average  $2 \times 6.5\mu$ .—CAROLINE T. RUMBOLD, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with Forest Products Laboratory, Forest Service, United States Department of Agriculture, Madison, Wisconsin.

*A Simple Method of Obtaining Pythium Cultures Free from Bacteria.*<sup>1</sup>—Bacterial contaminants, such as occur sometimes in newly isolated cultures of fungi, may be removed by acidifying the culture medium or by cutting

<sup>1</sup> Contribution No. 375 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Ontario, Canada.

out and transferring to a fresh medium uncontaminated portions of a culture growing on a solid medium. If, however, bacteria occur in a culture of *Pythium*, they are usually very persistent and frequently destroy the fungus. Because *Pythium* does not thrive on acidified media and because bacteria on neutral or alkaline media can multiply fast enough to keep pace with the growing tips of the hyphae, there is difficulty in obtaining uncontaminated *Pythium* cultures.

It is well known that fungi are able to grow within a solid medium as well as on the surface. The bacteria that contaminate fungal cultures, being usually obligate aerobes, are confined to the surface. If, therefore, the lateral spread of the bacterial colony on the surface of the medium is hindered or prevented, and the subsurface hyphae continue their growth, a pure culture of the fungus may be secured.

Brown<sup>2</sup> has made use of this knowledge in developing a method for the purification of fungal cultures. By inverting a portion of solid medium occupied by the contaminated culture over an unused part of the medium in the same Petri dish, he was able to obtain, within a few days, a pure culture of the fungus from the hyphae that had grown up through the inverted medium to its now upper surface.

Sometimes this method is not effective, particularly with some types of bacterial contaminants. Motile bacteria may escape through the film of liquid that separates the two layers of medium, particularly if the bacteria are of the rapidly multiplying, "Spreader" type; consequently, the culture may become recontaminated. Furthermore, if the medium be soft it is difficult to invert, and, if hard, cracking of the medium may occur over the inoculum and the bacteria may then spread through this avenue to the upper surface. To obviate these difficulties, another method, which has proved very satisfactory in culturing *Pythium*, has been developed. The same principle is involved. Instead, however, of inverting one layer of solid medium over another layer, the contaminated inoculum is covered with a sterile small glass disc. For this purpose a circular cover-glass, 20 mm. in diameter, has been found very satisfactory.

Melted potato-dextrose agar is poured into a sterile Petri dish. While the medium is yet soft, but cool enough so that the fungus is not killed, a small quantity of the contaminated *Pythium* culture is placed on the medium and immediately covered by the glass disc. The disc should be pressed down until it is in complete contact with the medium.

The glass disc prevents the lateral spread of the bacteria on the surface of the medium but does not hinder the growth of subsurface hyphae. If

<sup>2</sup> Brown, W. Two mycological methods. I. A simple method of freeing fungal cultures from bacteria. II. A method of isolating single strains of fungi by cutting out a hyphal tip. *Ann. Bot.* 38: 401-404. 1924.



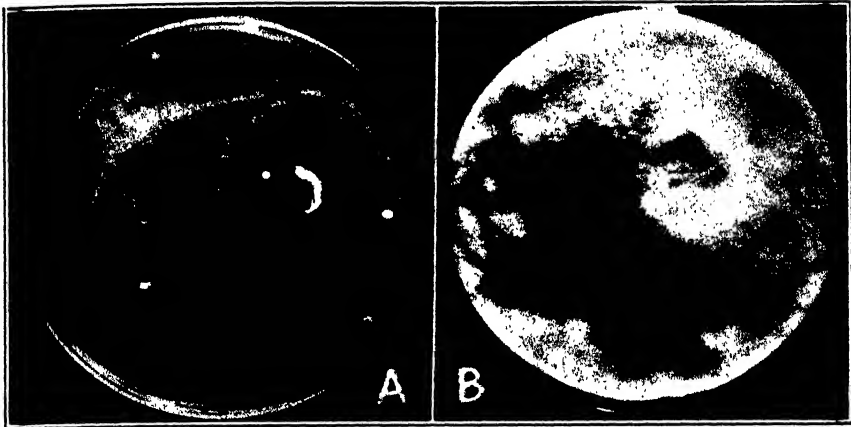


FIG. 1. Result of freeing a culture of *Pythium* from bacterial contaminants. A. Contaminated culture. B. Bacteria-free culture obtained by the glass-disc method.

the contaminated inoculum, thus transferred, has not been too adversely affected by the bacteria, the hyphae begin to emerge from beneath the disc within a few days. Transfers from the uncontaminated mycelium may then be made. The growth of the hyphae during the initial period may be observed by inverting a Petri dish and examining the culture under a microscope.

Figure 1 illustrates the efficacy of this method. The culture in Petri dish (A) is contaminated by bacteria. A portion of the culture has been transferred to Petri dish (B) and covered by the glass disc. After a few days the medium in the latter dish became overgrown by the fungus, particularly around the glass disc which is outlined by the matted hyphae. The whole culture in dish (B) outside of the disc is free from bacteria.—J. E. MACHACEK, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.

*Cell Degeneration in Relation to Sieve-tube Differentiation in Curly-top Beets. A Preliminary Note.*—Curly-top is primarily a disease of the phloem. This is the first tissue that shows degeneration and the only region that undergoes necrosis.<sup>1</sup> Moreover, a study of leaves of curly-top beets showed that the development of readily visible pathologic changes is definitely related to the differentiation of primary sieve tubes.<sup>2</sup> No cell degeneration could be observed in young leaves above the region of differentiation of primary sieve tubes, but, where these elements had matured,

<sup>1</sup> Esau, K. Pathologic changes in the anatomy of leaves of the sugar beet, *Beta vulgaris* L., affected by curly top. *Phytopath.* 23: 679-712. 1933.

<sup>2</sup> Esau, K. Ontogeny of the phloem in sugar beets affected by the curly-top disease. *Amer. Jour. Bot.* 1934. In print.



FIG. 1. A. Stele of a diseased root tip. B. Protophloem sieve tube with surrounding cells from a healthy root tip. C. Bundle from the third vascular ring of a diseased root. cc, companion cell; deg, degenerated cells; st, sieve tube. All  $\times 797$ .

the cells surrounding them developed abnormalities characteristic of curly-top phloem. Recent studies on the root confirmed these observations.

In the central cylinder of the primary root the first cells to mature are two protophloem sieve tubes located in transverse sections on opposite sides of the stele. Figure 1, B, shows a portion of the stele of a healthy root tip, the protophloem sieve tube appearing as though devoid of contents. The cells to the right of the sieve tube are young phloem cells, whereas the two large cells to the left belong to the pericycle.

In the root tips of diseased beets unmistakable signs of degeneration characteristic of curly top may first be observed in cells lying adjacent to the protophloem sieve tubes. These cells develop inclusion bodies and show hypertrophic and necrotic changes. Figure 1, A, represents a transverse section of the stele of a diseased root tip. The 2 apparently normal protophloem sieve tubes (st) are conspicuous by scarcity of contents. Degeneration had not yet progressed very far in the neighborhood of the sieve tube to the left, but 2 cells of the pericycle and 1 of the phloem showed a pronounced hypertrophy of nuclei. To the right, the cells adjacent to the sieve tube showed darkly stained disintegrating protoplasts. In later stages of infection degeneration spreads to other cells in the phloem region, causing the development of a peculiar hyperplastic tissue.<sup>2</sup>

When secondary growth occurs in the beet, successive concentric rings of vascular tissue develop in the fleshy portion of the root. In diseased plants phloem degeneration spreads to the newly formed rings. But here also sieve tubes that are apparently normal develop before degeneration sets in. Figure 1, C, shows a transverse section of a bundle taken from the third and youngest vascular ring in a young beet root. In the upper part of the figure is a sieve tube (st) that is the first to develop in this bundle. It is flanked by companion cells (cc) on 2 sides. The darkly stained structure above the sieve tube (deg 1) is a collapsed parenchyma cell that had undergone degeneration and necrosis. The parenchyma cell below the sieve tube (deg 2) shows a protoplast in an early stage of disintegration. The tissue layers farther below are represented by phloem, cambium and xylem. The phloem shows abnormalities that will not be described here.

The sieve tube seems to play a definite rôle in the initiation of degenerative changes in curly-top-diseased beets. Perhaps it represents the channel through which the virus is translocated to the newly developing parts of the root.—KATHERINE ESAU, University of California, Davis, California.

*Powdery Mildew of Flax in Minnesota.*—In September, 1933, the writer observed a powdery mildew on flax in the Coon Creek experimental peat plots near Anoka, Minnesota. The only varieties heavily infected were C. I. 669 and C. I. 743, although a few other varieties and hybrids were very lightly infected.

The mildew was found on the leaves, stems, and sepals. Perithecia were found only on the 2 heavily infected varieties. They were not numerous but often appeared in clusters on all of the plant parts attacked. The conidial stage was produced in the greenhouse 18 days after inoculation.

As far as the writer can determine, this is the first recorded appearance of flax mildew in North America, although it has been reported in Europe and Japan.<sup>1,2</sup> Škorić<sup>3</sup> first described the conidial stage, giving it the name, *Oidium lini*, n. sp. Homma, in 1927, according to her later publication,<sup>2</sup> reported a flax mildew that produced perithecia. She identified it as *Erysiphe polygoni* DC. Later she described the conidial stage of another mildew on flax that she considered identical with *Oidium lini* Škorić but

TABLE 1.—*Species identification and dimensions of conidia of flax mildew as given by various investigators.*

Investigator	Species	Length		Width	
		Range in $\mu$	Mean in $\mu$	Range in $\mu$	Mean in $\mu$
Homma . . . . .	<i>E. polygoni</i>	30.0–38.4		19.4–24.0	
Homma . . . . .	<i>O. lini</i>	26.4–36.0		12.0–14.4	
Škorić . . . . .	<i>O. lini</i>	26–41		12–15	
Salmon and Ware . . . . .	<i>E. polygoni</i> (probably)	31–40	34	12–16	14
Writer . . . . .	<i>E. cichoracearum</i>	23.1–33.5	$29.4 \pm 0.16$	12–18	$15.1 \pm 0.09$

distinctly different from *E. polygoni*. Badayeva<sup>4</sup> reported a powdery mildew, *E. cichoracearum* DC., as one of the most prevalent diseases on flax in Siberia.

The perithecia of the flax mildew collected in Minnesota were reddish brown, with numerous flexuous, darkened, simple appendages. The mean diameter of 50 perithecia was  $112.5 \pm 0.37\mu$  with a range of 84.7–142.7 $\mu$ . The perithecia usually contained 8 to 12 stalked asci. The mean width of

<sup>1</sup> Salmon, E. S., and W. M. Ware. The powdery mildew of flax. Gard. Chron. 82: 34–35. 1927.

<sup>2</sup> Homma, Yasu. On the powdery mildew of flax. Bot. Mag. (Tokyo) 42: 331–334. 1928.

<sup>3</sup> Škorić, Vladimir. Erysiphaceae Croatiae. Prilog fitopatološko-sistematskoj monografiji naših pepelnica. Glasnik za šumske Pokuse. (Annales pro Experimentis Foresticis) Zagreb. 1: 52–118. 1926.

<sup>4</sup> Badayeva, P. K. O bolezniokh l'na v Sibiri. Morbi Plantarum, Leningrad, 19: 192–199. 1930. (Abs.) Rev. Appl. Myc. 10: 459. 1931.

Paper No. 1237 of the Journal Series of the Minnesota Agricultural Experiment Station.

100 asci was  $27.9 \pm 0.20 \mu$  and the range 21.3–34.6 $\mu$ . The asci contained predominantly 2 ascospores, rarely 1 or 3. The mean width of 100 ascospores was  $11.6 \pm 0.07 \mu$ , and the range was 9.4–14.6 $\mu$ . The mean length was  $21.4 \pm 0.13 \mu$  with a range of 15.9–27.3 $\mu$ . On the basis of these characters and measurements the mildew was identified as *Erysiphe cichoracearum* DC.

Measurements of the conidia obtained by the writer are compared in table 1 with the conidial measurements obtained by Homma, by Škorić, and by Salmon and Ware. The range in width of the conidia of the Minnesota mildew is outside that given by Homma for *Erysiphe polygoni* but agrees with that given by Škorić and Homma for *Oidium lini*, and by Salmon and Ware for the mildew that they described. The range in length is different from that for *E. polygoni* but very similar to that given by Škorić and Homma for *O. lini*. The range in length of conidia reported by Salmon and Ware is approximately the same as that given by Homma for *E. polygoni*. However, since the width of conidia of powdery mildews is considered more constant than the length, it seems more likely that the mildew described by Salmon and Ware is the same as that described in this paper. The conidial stage of the mildew found in Minnesota appears to be the same as *O. lini* described by Škorić and Homma. On the basis of conidial and perithecial characters the mildew is *E. cichoracearum*.—C. C. ALLISON, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

*Olive Knot on Olea chrysophylla*.—*Olea chrysophylla* Lam. has been distributed at different times by the United States Department of Agriculture. It is native to the highlands of East Africa and differs from *O. europea* L. in having leaves whose dorsal surface is yellowish or drab instead of light green (almost white).

*Olea chrysophylla* has been successfully inoculated with the olive-knot organism, *Bacterium savastanoi*. (Fig. 1.). The resulting knots, however, differ in appearance from those occurring on *O. europea*. They are smaller, less globose, and rougher or more irregular in form. In appearance they resemble more those produced by *Bact. savastanoi* on certain hosts<sup>1</sup> allied to the olive, such as Fraxinus, Forestiera, and Osmanthus. They also have a general resemblance to galls produced by *Bact. tumefaciens*, but *Bact. tumefaciens* is not known to make galls on *O. europea*, the source from which these original cultures were isolated.

The inoculations on the 2 hosts were made by using 2 different cultures of *Bacterium savastanoi*, and a period of 13 months elapsed between the 2 series of inoculations.

<sup>1</sup> Smith, C. O. Pathogenicity of the olive knot organism on hosts related to the olive. *Phytopath.* 12: 271–278. 1922.



FIG. 1. Knots on *Olea chrysophylla* 20 months after inoculation marked by metric measure. Knots above on *Olea europea* after 7 months.

The knots on *Olea chrysophylla* were photographed 20 months and those of *O. europea* 7 months after inoculation. By that time these knots should have reached their final size and form and would show any difference in character of knot. The differences as given are believed to be due to a difference in growth responses between the causal organism and the individual hosts.

The causal organism was reisolated from the artificial knots on *Olea chrysophylla*, and reinoculations into *O. europea* and *O. chrysophylla* produced knots typical for each of the two hosts.—CLAYTON O. SMITH, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

*Color in Relation to Virulence in Aplanobacter michiganense.*—Since April, 1931, studies have been in progress on color variation in *Aplanobacter michiganense*, the cause of bacterial canker of tomatoes,<sup>1,2</sup> with the object of discovering the significance of these variations and the extent to which they may be amenable to experimental control.

Both the serial culture method of Hadley,<sup>3</sup> using beef bouillons adjusted

<sup>1</sup> Bryan, Mary K. An albino strain of *Aplanobacter michiganense*. (Abst.) Phytopath. 20: 141. 1930.

<sup>2</sup> Bryan, Mary K. Color variations in *Aplanobacter michiganense*. Phytopath. 21: 559. 1931.

<sup>3</sup> Hadley, P., E. Delves, and J. Klimek. The filtrable forms of bacteria: I. A filtrable stage in the life history of the Shiga dysentery bacillus. Jour. Inf. Dis. 48: 1-159. 1931.

to pH 6.0, pH 7.0 and pH 8.0, and the technic described by Quirk<sup>4</sup> for the production of rough and smooth colonies, modified to suit the needs of this slow-growing organism, were employed.

Preliminary experiments with laboratory stock cultures of the *yellow*, *white* and *pink* forms showed the pink one to be most readily affected by environmental conditions. Both this earlier work and later experiments with a *single-cell* strain of the *pink* form indicate (1) that the pH of the media, the age of the culture, the amount of dilution to which it is subjected, and the age of the dilutions when plated all may be factors in controlling the form and color of colonies obtained; (2) that color changes bear a definite relation to the rough and smooth types or stages in the life of a culture and (3) that *color* as well as colony form is linked with degree of *virulence*.

The writers have repeatedly obtained experimentally the yellow and white forms from the pink one and have consistently found them both to be markedly more infectious than the parent culture, the pink form in many instances being nonvirulent.

Within any one color strain both rough and smooth colonies occur and, when working with the pink strain, a wide range of color and form has been found, including intermediates that combine the qualities of roughness and smoothness and appear to represent blends of "pink," yellow and white to form salmons, buffs, and oranges of different intensities.

An article treating of certain phases of the work is planned in the near future.—EDNA H. FAWCETT and MARY K. BRYAN, Bureau of Plant Industry, Washington, D. C.

*The Host Specificity of Septoria petroselini and S. apii-graveolentis.*—The question of specificity of two species of *Septoria* affecting celery and facts concerning their distinction from the parsley *Septoria* was reported by the author<sup>1</sup> in 1932. In that work neither of the celery forms, although they had both been called *Septoria petroselini*, were found to infect parsley, thus for purposes of clarifying nomenclature were concluded to be specific to celery. Aside from the desirability of testing *S. petroselini* on celery, a culture could not be obtained at that time.

Recently, cultures of *Septoria petroselini* and *S. apii-graveolentis* were sent to the writer by Dr. Johanna Westerdijk from the Central-Bureau voor Schimmelcultures of Baarn, The Netherlands. Two lots of twenty celery plants were inoculated respectively with the organisms and infection occurred uniformly on the plants inoculated with *S. apii-graveolentis*. The

<sup>4</sup> Quirk, Agnes J. Pure smooth and rough colony types at will. *Science* n. s. 74: 461. 1931.

<sup>1</sup> *Phytopath.* 22: 791-812. 1932.

same experiment was repeated using parsley plants and infection resulted only on the plants inoculated with *S. petroselini*. Thus it is entirely clear that these two organisms are specific to their respective hosts and the separation of the celery Septorias from *S. petroselini* further justified.—L. C. COCHRAN, Mich. Agr. Exp. Sta., East Lansing, Mich.



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## THE HOST RANGE AND BEHAVIOR OF THE ORDINARY TOBACCO-MOSAIC VIRUS<sup>1</sup>

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Although tobacco mosaic was described as early as 1886, the probable host range of the virus concerned has remained obscure. With the discovery of mosaic diseases in various other species, a strong tendency persisted on the part of some to attribute these to the virus of tobacco mosaic, or a somewhat modified form of this virus. Even though good evidence of the existence of different mosaic viruses began to accumulate about 1916, it was hardly sufficient for a decade to counteract this tendency. The more recent evidence of virus specificity based upon additional methods of identification has now enabled more reliable determination of host ranges to be made.

The ordinary tobacco-mosaic virus (*tobacco virus 1*) (23) has, in recent years, generally been regarded as limited to the Solanaceae. Confirmed evidence has existed for some time, however, that this virus is transmissible to 2 genera outside this family. This has suggested the possibility of a wider host range and the need of more complete information on this subject in relation to both the classification of plant viruses and the development of control measures. With this purpose in view a systematic study of the host range was undertaken, selecting for the tests widely separated plant species. The results secured show that *tobacco virus 1* is capable of developing in certain species through a wide range of plant families, with no appreciable modification of the virus itself in the different hosts.

### REVIEW OF LITERATURE

Mayer (28) first reported that the tobacco-mosaic disease could be transmitted mechanically from infected to healthy tobacco plants. The first proof that tobacco-mosaic virus is transmissible to other solanaceous genera (*i.e.*, *Lycopersicon*) was reported by Clinton (6) in 1908. Allard (1), in 1914, re-

<sup>1</sup> This investigation was conducted under allotments from the University of Wisconsin Research Fund.

<sup>2</sup> The writer wishes to acknowledge his appreciation of the helpful advice and direction of the work by Dr. James Johnson, and the timely suggestions of Dr. Ismé A. Hoggan.

ported tobacco mosaic as communicable to a number of other solanaceous species. All of his efforts, however, to communicate the disease to plants of families other than the Solanaceae gave negative results. The following plants were inoculated by the most rigorous methods without any visible symptoms of the disease appearing: sheep sorrel, white clover, red clover, peanut, soy bean, cauliflower, peppergrass, rib-grass, dead nettle, wood sorrel, garden lettuce, and pokeweed. Similarly, Duggar and Armstrong (10) and Johnson (23) failed to transmit the tobacco-mosaic virus to *Phytolacca decandra* L. Clinton and McCormick (7) and E. M. Johnson (19) also reported failure to transmit this virus to certain other non-solanaceous plants.

Following the work of Allard, numerous species were reported, however, as showing mosaic symptoms. In 1922 Dickson (8) reviewed the literature and listed 96 species on which mosaic had been found. Of these species, 30 belong to the Solanaceae, 20 to the Cucurbitaceae, 18 to the Leguminosae, and 7 to the Gramineae, the other 21 hosts being distributed through 17 families. Dickson recognized the possibility that there might be different mosaic diseases involved; however in his review he made no distinction, listing only those plants on which mosaic had been found.

Elmer (11), in 1922, reported that cross-inoculation experiments showed the mosaic diseases of the Cucurbitaceae, Solanaceae, and Leguminosae to be intertransmissible. In 1925 he further reported (12) the mosaic virus transmissible among species belonging to different families and orders. Fifteen interfamilial and 11 interorder transmissions were obtained. Results of a similar nature were reported by Palm (30), Palm and Jocheims (31), Walker (36), and Doolittle and Walker (9). It is evident that at this time the specificity of viruses was not generally recognized or accepted, even though somewhat prior to and during this period evidences of differences in host range and symptomatology were being presented by Allard (2), Schultz and Folsom (33), Fernow (13), and Johnson (20, 21).

The danger of confusion of plant viruses in host-range studies is evident from Johnson's (23) description of 11 different viruses on tobacco, as differentiated on the basis of several factors applicable as tests

Fernow (13), in 1925, described his mosaic A, which, as nearly as can be determined, was due to the ordinary tobacco-mosaic virus, as transmissible to *Martynia louisiana*, Mill. of the family Martyniaceae. The susceptibility of this nonsolanaceous plant to this virus was confirmed later by Beale (3), and Hoggan (15). This is, then, the first nonsolanaceous species definitely shown to be susceptible to the ordinary tobacco-mosaic virus. In 1930 Price (32) tested 77 commercial varieties of *Phaseolus vulgaris* L. and found that certain varieties develop local necrotic lesions when juice, con-

taining virus of ordinary tobacco mosaic, is rubbed onto the upper surfaces of the leaves, thus establishing this species, also, as a host.

#### MATERIALS AND METHODS

One hundred and twenty-one species representing 40 widely separated families were included in this study. The selection of these species was based first upon securing a wide family distribution, and secondly upon the availability of seed; hence the use of a number of common crop, ornamental, and weed plants. Previous mention in literature of susceptibility of certain species to mosaic, especially to tobacco mosaic, rendered the inclusion of such species desirable. The majority of seed was obtained from the following seed companies: L. L. Olds, Madison, Wisconsin; H. G. Hastings, Atlanta, Georgia; and Theodore Payne, Los Angeles, California. The weed seeds were secured from A. L. Stone, Agronomy Department, University of Wisconsin. All species used were, as nearly as could be determined, correctly named. The seed was sown in greenhouse flats and the seedlings transplanted to fertile compost soil in 4-in. pots.

The ordinary tobacco-mosaic virus (*tobacco virus 1*) was the same as that used in a previous investigation by Johnson and Grant (25). The original source was maintained by frequent direct inoculations to healthy young tobacco plants. These stock plants for the virus were kept in a special chamber and precautions were taken to avoid contamination with other viruses. All plants tested for susceptibility were inoculated with extracts obtained from these stock plants.

The experiments were carried on during the fall, winter, and spring in 2 greenhouses, one of which was maintained at a temperature of 80° to 90° F., and the other at 65° to 70° F. During the summer of 1932 infected plants were grown out of doors. This afforded an opportunity to study symptom expression of various hosts under field conditions.

Holmes' wiping method of inoculation (16) was employed throughout the present investigation. Leaf material was crushed in a mortar and the extracted juice strained through cheesecloth. A cheesecloth pad, saturated with extract, was rubbed over the entire upper surface of the leaves inoculated. The lower leaves only of the young plants were inoculated and the plants were then allowed to grow for 2 to 3 weeks or more to allow sufficient time for the distribution of the virus in case of infection. Inoculations with extracts from the noninoculated leaves were then made to *Nicotiana tabacum* L. and *N. glutinosa* L. to determine the presence or absence of the virus. All plants inoculated with the virus were tested for susceptibility in this manner. Furthermore, additional plants of susceptible species were repeatedly tested, and the symptom expression noted in comparison with noninoculated controls.

The distribution and relative concentration of the virus were determined by making separate extracts from top, middle, and basal leaves and inoculating to 5 leaves of *Nicotiana glutinosa* plants. The number of lesions appearing after 3 to 5 days was then recorded. The methods used for determining the thermal death point, tolerance to dilution, and longevity *in vitro* of virus obtained from different hosts were the same as those described by Johnson (23, 25). In this investigation it was necessary to take special precautions to obtain extracts with as high virus content as possible. Details of other methods used are presented in the text.

#### EXPERIMENTAL RESULTS

##### Host Range

The host range studies were limited to nonsolanaceous species. Over 2,000 flowering plants, representing 40 families, 104 genera, and 121 species, were inoculated with the ordinary tobacco-mosaic virus. A summary of the results secured is given in table 1. No infection was obtained on 76 per cent of the species tested. It is difficult to attach any great significance to this fact, however, since proof that a particular species is immune from the virus would no doubt require a larger number of trials under a greater variety of environmental conditions than was made in these tests. The main difficulties encountered in the tests were the consequence of the erratic spread of the virus in some hosts, rendering determination of infection laborious, and the different environmental conditions required for satisfactory vegetative development of some species. While the negative results obtained by the inoculation of species grown under similar environmental conditions are of some interest in terms of percentage of immune species, they are not believed to be of sufficient value to list in full in this paper.

No infection was obtained on any monocotyledonous plants, although 16 different species representing 5 families were tested. Special attention was given to the inoculation of plants in the family Gramineae, such as: corn, rye, wheat, barley, oats, various millets, sudan grass, sorghum, feterita, and sugar cane.

One hundred and five dicotyledonous species representing 35 families were inoculated. Negative results were obtained on all species tested in 21 of these families. In some of these families several species were used; as, for example, 6 species in the Labiatae, 6 in the Cucurbitaceae, and 4 in the Malvaceae. On the other hand, positive infection resulted from the inoculation of 29 species included in the following 14 families: Polygonaceae, Chenopodiaceae, Aizoaceae, Ranunculaceae, Cruciferae, Leguminosae, Umbelliferae, Convolvulaceae, Polemoniaceae, Hydrophyllaceae, Boraginaceae, Scrophulariaceae, Martyniaceae, and Compositae.

In most of these families infection was not secured on all species tested.

TABLE 1.—*Results of inoculating certain species of various plant families with the ordinary tobacco-mosaic virus*

Family name	Number species tested	Number plants inoculated	Number species infected
Gramineae	12	390	0
Araceae	1	5	0
Commelinaceae	1	15	0
Liliaceae	1	10	0
Cannaceae	1	5	0
Polygonaceae	2	79	1
Chenopodiaceae	2	147	2
Amaranthaceae	2	24	0
Nyctaginaceae	1	5	0
Aizoaceae	1	20	1
Portulacaceae	1	10	0
Carophyllaceae	1	5	0
Ranunculaceae	3	44	1
Papaveraceae	1	10	0
Cruciferae	7	180	2
Leguminosae	13	96	1
Geraniaceae	1	5	0
Tropaeolaceae	1	5	0
Euphorbiaceae	1	5	0
Balsaminaceae	1	5	0
Malvaceae	4	65	0
Violaceae	1	5	0
Lythraceae	1	15	0
Onagraceae	1	21	0
Umbelliferae	2	37	1
Plumbaginaceae	1	10	0
Apocynaceae	1	14	0
Convolvulaceae	4	68	2
Polemoniaceae	2	8	1
Hydrophyllaceae	5	90	5
Boraginaceae	2	30	1
Verbenaceae	1	20	0
Labiatae	6	67	0
Scrophulariaceae	7	171	6
Martyniaceae	1	10	1
Plantaginaceae	1	5	0
Valerianaceae	1	10	0
Cucurbitaceae	6	60	0
Campanulaceae	1	10	0
Compositae	19	363	4
	121	2144	29

In the Compositae only 4 out of 19, in the Leguminosae 1 of 13, and in the Convolvulaceae 2 of the 4 species tested were found susceptible to infection. In the family Hydrophyllaceae, however, all 5 species inoculated became infected.

The 29 species found susceptible to infection are: buckwheat, *Fagopyrum esculentum* Gaertn.; garden and sugar beet, *Beta vulgaris* L.; Swiss chard, *B. vulgaris* var. *cicla* L.; spinach, *Spinacia oleracea* L. var. King of Denmark; New Zealand spinach, *Tetragonia expansa* Murr.; larkspur, *Del-*

*phinium consolida* L.; mustard, *Brassica alba* Rabenh.; turnip, *B. rapa* L.; bean, *Phaseolus vulgaris* L. var. Green Pod Refugee; carrot, *Dacus carota* var. *sativa* DC.; morning glory, *Ipomoea tricolor* Cav.; cypress vine, *Quamoclit pennata* Bojer; phlox, *Phlox drummondii* Hook.; California blue bell, *Phacelia whittlavia* Gray, *P. campanularia* Gray, *P. grandiflora* (Benth.) Gray, *P. tanacetifolia* Benth., *P. parryi* Torr.; Chinese forget-me-not, *Cynoglossum amabile* Stapf. and Drummond; mullein, *Verbascum thapsus* L.; Kenilworth ivy, *Linaria cymbalaria* (L.) Mill.; snapdragon, *Antirrhinum majus* L.; foxglove, *Digitalis purpurea* L.; Maryland figwort, *Scrophularia marylandica* L.; beard-tongue, *Penstemon barbatus* Roth.; unicorn plant, *Martynia louisiana* Mill.; zinnia, *Zinnia elegans* Jacq.; French marigold, *Tagetes patula* L.; cape marigold, *Dimorphotheca aurantiaca* DC.; tassel flower, *Emilia sagittata* (Vahl.) DC.<sup>3</sup>

### Symptom Expression and Type of Infection

The descriptions of symptoms and type of infection, as typical for the 29 nonsolanaceous hosts, must be, in a sense, only relative. The natural variations in environment and other circumstances, such as age and vigor of the host plant inoculated, may modify both the distribution of the virus and the symptom expression. Certain of the hosts, however, repeatedly developed remarkably specific symptoms and virus distribution and may be used to illustrate the various types of infection secured (Table 2).

California blue bell, *Phacelia whittlavia*, showed systemic infection very similar in type to that generally recognized as occurring with ordinary tobacco mosaic on tobacco, *Nicotiana tabacum*. Mottling, malformation, and chlorosis were the predominating manifestations (Fig. 1, A, B.). In other species, such as larkspur and Chinese forget-me-not, there was occasionally more stunting of the plant and considerable necrosis (Fig. 2, B.). The virus was generally found distributed throughout the plant, and these hosts were considered, therefore, as being systemically infected. Spinach, also, was placed with this group of hosts on the basis of symptom expression.

In contrast to the above species, which were found to be systemically infected, others showed a localized type of infection, the virus apparently not spreading much beyond the inoculated portion. Garden beet, Swiss chard, and New Zealand spinach first exhibited symptoms of yellowing only at the point of entrance of the virus. These yellow areas usually extended until the entire leaf was chlorotic. The leaf finally became necrotic and sometimes dropped off. The symptoms appearing on Green Pod Refugee

<sup>3</sup> Credit should be given to Dr. Maurice B. Lindford for being first to secure infection with the ordinary tobacco-mosaic virus on *Emilia sagittata*, while performing certain experiments at Wisconsin during the summer of 1931.

TABLE 2.—A comparison of type infection and symptom expression obtained with ordinary tobacco mosaic on nonsolanaceous host plants

Type of infection	Host species	Host family	Number of plants <sup>a</sup>	Symptom expression <sup>b</sup>
Systemic	<i>Phacelia whittlavia</i> (California blue bell)	Hydrophyllaceae	30	M-MF-s
			27	
	<i>Phacelia parryi</i>	"	20	M-MF-s
			20	
	<i>Phacelia campanularia</i>	"	10	M-MF
			10	
	<i>Phacelia grandiflora</i>	"	20	Y-M mf
			20	
	<i>Phacelia tanacetifolia</i>	"	10	Y-m-n
			10	
	<i>Delphinium consolida</i> (larkspur)	Ranunculaceae	28	Y-M-n
			28	
	<i>Cynoglossum amabile</i> Chinese forget-me-not)	Boraginaceae	25	M-mf-s
			25	
Local	<i>Martynia louisiana</i> (unicorn plant)	Martyniaceae	10	M-mf-s
			7	
	<i>Spinacia oleracea</i> (spinach)	Chenopodiaceae	55	Y-M-s-n
			44	
	<i>Beta vulgaris</i> (garden beet)	Chenopodiaceae	35	Y-N
			30	
	<i>Beta vulgaris</i> (sugar beet)	"	5	Y-N
			5	
	<i>Beta vulgaris</i> var. <i>ciela</i> (swiss chard)	"	52	Y-N
			44	
	<i>Tetragonia expansa</i> (New Zealand spinach)	Aizoaceae	20	Y-N
			20	
	<i>Phaseolus vulgaris</i> (green pot refugee bean)	Leguminosae	5	N
			5	
Erratic	<i>Emilia sagittata</i> (tassel flower)	Compositae	45	MS-m
			37	
	<i>Linaria cymbalaria</i> (Kenilworth ivy)	Scrophulariaceae	35	Ms-y-m-mf
			29	
	<i>Phlox drummondii</i> (phlox)	Polemoniaceae	18	M-y
			12	
	<i>Digitalis purpurea</i> (foxglove)	Scrophulariaceae	25	Y-m
			25	
	<i>Verbascum thapsus</i> (mullein)	"	25	Y-m
			12	
	<i>Ipomoea tricolor</i> (morning glory)	Convolvulaceae	39	Y-n
			12	
	<i>Tagetes patula</i> (French marigold)	Compositae	55	Y-m-n
			39	
	<i>Zinnia elegans</i> (zinnia)	"	55	Y-m
			23	
	<i>Fagopyrum esculentum</i> (buckwheat)	Polygonaceae	64	M-MF-n-s
			46	

TABLE 2.—(Continued)

Type of infection	Host species	Host family	Number of plants <sup>a</sup>	Symptom expression <sup>b</sup>
Undetermined	<i>Antirrhinum majus</i> (snapdragon)	Scrophulariaceae	35 6	MS-Y
	<i>Penstemon barbatus</i> (beard-tongue)	“	44 8	MS-Y
	<i>Scrophularia marylandica</i> (Maryland figwort)	“	9 5	MS-y
	<i>Quamoclit pennata</i> (cypress vine)	Convolvulaceae	21 3	Y-n
	<i>Daucus carota</i> var. <i>sativa</i> (carrot)	Umbelliferae	25 1	M-n
	<i>Brassica alba</i> (mustard)	Cruciferae	65 15	M-N
	<i>Brassica rapa</i> (turnip)	“	20 5	M-N
	<i>Dimorphotheca aurantiaca</i> (Cape marigold)	Compositae	20 6	N-mf

<sup>a</sup> In this and succeeding tables, upper figure represents number of plants inoculated; lower figure, number of plants infected.

<sup>b</sup> M—mottling; MF—malformation; S—stunting; Y—yellowing; N—necrosis; MS—masked symptoms. Small type indicates mild form of symptoms.

bean were essentially of the same type of necrotic lesion as described by Price (32).

During the course of this investigation other species were found in which the type of infection could not be definitely classed as either systemic or localized. The uncertain behavior of this group of species with respect to infection has led us to place it in a third category designated as “erratic.” Since this study was undertaken Holmes (18) also has reported that in certain solanaceous hosts the virus spreads erratically from the inoculated leaf. Essentially the same method as that used by Holmes has been applied in the study of certain of the nonsolanaceous hosts characterized by irregular reaction to inoculation. Leaves from top, middle, and basal portions of such plants were crushed separately in mortars and the extracts, so obtained, used for inoculation to *Nicotiana glutinosa*. The total number of lesions appearing after 3 to 5 days was counted and the data are presented in table 3. These counts not only serve to show the presence or absence of the virus in the various parts of the plant, but indicate as well its relative concentration.

Ordinarily, tassel flower and Kenilworth ivy did not show any symptoms, though some mottling and chlorosis could be induced by partial defoliation. The results obtained on virus distribution indicated that the virus was present in higher concentration in the basal than in the top leaves.



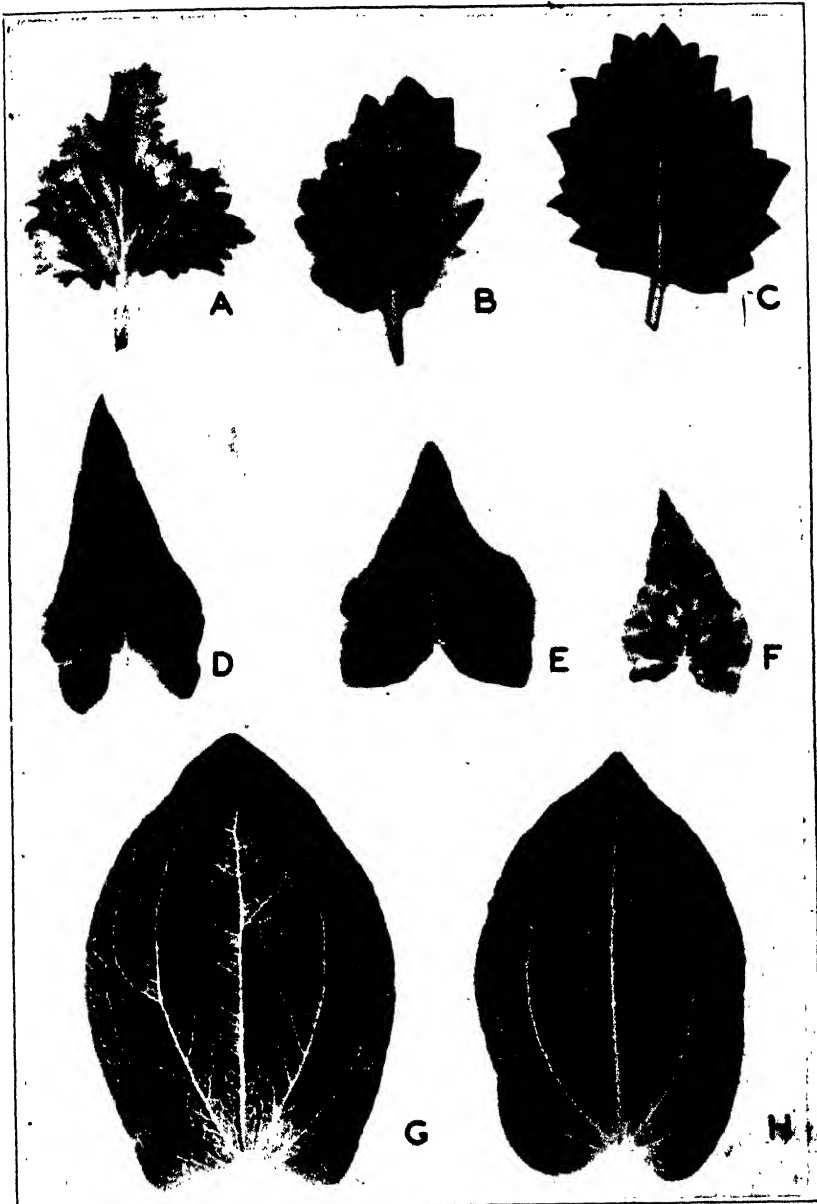


FIG. 1. Leaf symptoms of the ordinary tobacco mosaic on certain nonsolanaceous species. A. and B. Infected leaves of *Phacelia whittlaria*, showing mottling, malformation, and chlorosis. C. Leaf from noninfected control. D-F. Infected leaves of *Fagopyrum esculentum* (buckwheat). G. Infected leaf of *Zinnia elegans*, showing chlorotic spots resulting from spread of the virus from lower inoculated leaves. H. Leaf from noninfected control.



FIG. 2. Symptoms of ordinary tobacco mosaic on certain nonsolanaceous plants. A. Healthy *Cynoglossum amabile* (Chinese forget-me-not). B. Early stunting and malformation due to virus infection. C. Healthy *Tagetes patula* (French marigold). D. Infected plant showing stunting and malformation of leaflets.

Foxglove showed definite symptoms of chlorosis on the inoculated leaves. The youngest leaves did not at first show symptoms, although, as they became older, yellow spots appeared which coalesced to form either chlorotic or mottled areas. The distribution and concentration of the virus in this species seemed to be quite definitely related to the symptom expression. No virus was detected in the youngest leaves, while the highest concentrations were found in the older leaves.

In morning glory and mullein, the virus apparently had difficulty in

TABLE 3.—*Comparison of tobacco-mosaic virus content of basal, middle, and top leaves from various plants by means of inoculation to Nicotiana glutinosa*

Infected host	Period following inoc. (weeks)	Av. number of lesions on 5 leaves		
		From basal leaf	From middle leaf	From top leaf
<i>Emilia sagittata</i> (tassel flower)	3	198	185	0
	3	205	193	0
	3	421	264	0
	3	271	342	2
	10	136	4	0
	10	417	18	32
<i>Linaria cymbalaria</i> (Kenilworth ivy)	8	437	425	370
	9	240	210	67
	9	164	231	116
	9	184	27	4
	11	326	3	2
	11	258	205	7
<i>Digitalis purpurea</i> (foxglove)	7	610	340	0
	8	800	205	0
	8	456	700	0
	8	650	177	0
	8	346	189	0
	16	315	19	0
<i>Tagetes patula</i> (French marigold)	1	265		0
	4	532	499	519
	4	670	451	556
	4	650	0	0
	7	128	0	0
	8	373	204	292
	8	184	0	0
	8	238	226	282
<i>Zinnia elegans</i> (zinnia)	3	162	.....	0
	3	7	.....	0
	4	46	.....	0
	4	223	.....	133

passing from the inoculated leaves. The inoculated portion of the plant usually became chlorotic, and when the virus did pass from this portion it was found only in localized yellow spots or small areas on leaves immediately above. Older plants did not show symptoms on the top or youngest leaves.

In French marigold the virus was sometimes confined to the basal leaves and at other times was found in relatively high concentrations in all parts

of the plant tested. Although the symptom expression was variable, the inoculated leaves usually showed some chlorosis. The plant might be stunted in development and malformed, as shown in Figure 2, D, or it might develop mild symptoms of scattered chlorotic and necrotic spots. Leaf development in these spots was partially inhibited and, as a result, the leaf was slightly distorted.

The distribution of the virus in zinnia was particularly variable; it might be localized, causing only local lesions, or it might spread to other leaves, resulting in the appearance of small round yellow spots (Figure 1, G.). The spots sometimes remained yellow for a few days and finally disappeared or sometimes coalesced to form a large yellow blotch, which either remained the same color or changed to a light green.

In buckwheat the virus apparently passed readily from the inoculated leaf and, at first, produced what appeared to be typical systemic infection (Fig. 1, D-F), though, as the plant continued to grow, the symptoms became less marked in the new growth and the concentration of the virus decreased. Occasionally, necrosis developed in the stem and produced a lesion that caused distortion or death of the entire plant.

In this group of plants typified by erratic infection it was found that the symptom expression was extremely variable, ranging from masked symptoms to yellowing with some mottling and necrosis. The results secured from the testing of various plant parts showed definitely that there was considerable variation in the distribution of the virus. The significance of this is perhaps most strikingly brought out by comparison with results obtained by Holmes (17), who studied the local and systemic increase of tobacco-mosaic virus on *Nicotiana tabacum*.

A few of the nonsolanaceous plants inoculated exhibited a considerable degree of resistance to infection. In some species the percentage of plants infected was small, rendering determination of the type of infection difficult, although the presence of the virus in the infected plants was definitely proved by reinoculation to *Nicotiana glutinosa* and *N. tabacum*. During the early part of this investigation several series of snapdragon plants were inoculated, but infection was secured on only two. These plants did not develop definite symptoms. The presence of the virus in some shoots and not in others was determined by reinoculation to *N. glutinosa*. One of the infected snapdragon plants was grown in the garden during the summer of 1932 and seed was obtained from this plant. When this seed was planted and the young plants inoculated, 4 of 10 tested became infected.

Observations made on 8 infected beard-tongue plants indicated that these behaved in a manner similar to snapdragon. Cypress vine showed some yellowing of the basal leaves, and these contained virus, but the tops were apparently virus-free.

The study of the type of infection obtained in Maryland figwort was limited by the available seed supply. However, the plants tested showed that the virus might reach the top, though it was not found there in such high concentration as in the lower leaves. There were no definite symptoms, although the lower leaves finally became yellow.

One carrot plant, which became infected, showed mottling in all the leaves and these later became necrotic. This plant was inoculated in the greenhouse and later transplanted to the garden. The presence of tobacco-mosaic virus in this plant was tested several times by reinoculation to both tobacco and *Nicotiana glutinosa* and positive results were obtained in each case. Repeated attempts to secure further infection on carrots failed.

Mustard and turnip included in 3 separate trials during the winter of 1931 developed symptoms of marked mottling followed by complete necrosis of the plants. The seed source used in these first trials was exhausted and the plants grown from other seed have never shown infection. The infections secured on cape marigold were the result of inoculations made in a number of different trials. The symptoms consisted of necrosis of the mid-ribs of the leaves with subsequent curling and dwarfing of the plant.

Since, in most cases, the number of plants infected in this latter group of species was so low as to preclude satisfactory determination of type of infection, these have been listed in table 2 under "undetermined" type of infection.

The low percentage of infection obtained from repeated inoculation of certain nonsolanaceous hosts is of considerable interest and significance. Evidently, no "true symptomless carriers" of the virus were found. In every case where the virus was recovered from the species inoculated, symptoms were expressed at some stage of development, although partial masking of symptoms was not uncommon in many species.

#### Properties of the Virus in Different Hosts

*Thermal Death Point.* The thermal death point or inactivation point of ordinary tobacco-mosaic virus from tobacco generally is accepted as being close to 90° C. for a 10-minute treatment. Some variation is to be expected as a consequence of various circumstances, even though the conditions of the trials are made as constant as possible. According to McKinney (29) higher dilutions of the virus may lower the thermal death point several degrees. In a previous investigation Johnson and Grant (25) determined the thermal death point of the tobacco-mosaic virus in undiluted extracts from various solanaceous plants in which the virus occurred systemically. They concluded that in the species tested the thermal death point for this virus was just below 90° C., but that certain host species concerned might vary it as much as 5° C.

In the present investigation, due to the fact that the virus spread erratically in certain host plants, it was necessary to determine the location of the virus in these plants so that the possibility of dilution with the plant juice would be reduced to a minimum. The inoculated portion of the plants was not employed as a source of inoculum in any of the tests reported in tables 4 and 5. All the data on inoculation to *Nicotiana glutinosa*

TABLE 4.—Results of thermal death-point determinations on the tobacco-mosaic virus from different host species following inoculation to *Nicotiana tabacum*

Source of inoculum	Inoc. control	Temperature °C. (10 min.)			
		80	85	90	95
<i>Nicotiana tabacum</i> (tobacco)	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{2}$	$\frac{10}{0}$
<i>Phacelia whittlavia</i> (California blue bell)	$\frac{15}{15}$	$\frac{15}{14}$	$\frac{15}{10}$	$\frac{15}{0}$	$\frac{15}{0}$
<i>Spinacia oleracea</i> (spinach)	$\frac{20}{13}$	$\frac{20}{19}$	$\frac{20}{2}$	$\frac{20}{5}$	$\frac{20}{0}$
<i>Digitalis purpurea</i> (foxglove)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{0}$	$\frac{15}{0}$
<i>Fagopyrum esculentum</i> (buckwheat)	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{9}$	$\frac{10}{0}$	$\frac{10}{0}$
<i>Zinnia elegans</i> (zinnia)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{6}$	$\frac{15}{0}$	$\frac{15}{0}$
<i>Linaria cymbalaria</i> (Kenilworth ivy)	$\frac{15}{12}$	$\frac{15}{13}$	$\frac{15}{10}$	$\frac{15}{6}$	$\frac{15}{0}$
<i>Tagetes patula</i> (French marigold)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{1}$	$\frac{15}{0}$

presented in table 5 are based upon inoculation with the same samples of extract as those used to inoculate *N. tabacum*, as shown in table 4. However, not all the treated extracts inoculated to tobacco (Table 4) were inoculated to *N. glutinosa* because of the limited amount of space available in the greenhouse and the lack of sufficient *N. glutinosa* plants at the time the tests were made.

In table 4, using the number of *Nicotiana tabacum* plants infected as a criterion, it is shown that in extracts from *Phacelia whittlavia* infected with tobacco mosaic the virus was not greatly inactivated at 85° C., but was destroyed at 90° C. Similar results were secured with foxglove, buckwheat, and zinnia. In the case of spinach, Kenilworth ivy, and French marigold, the virus was not entirely inactivated at 90° C., a result similar to that frequently obtained with virus extract from tobacco.

TABLE 5.—Results of thermal death-point determinations on the tobacco-mosaic virus from different host species following inoculation to *Nicotiana glutinosa*

Source of inoculum	Number trials	Av. number of lesions on 5 leaves				
		Inoc. control	Temperature °C. (10 min.)			
			80	85	90	95
<i>Nicotiana tabacum</i> (tobacco)	2	602	433	264	1	0
<i>Phacelia whittlavia</i> (California blue bell)	2	208	47	9	0	0
<i>Spinacia oleracea</i> (spinach)	3	22	69	1	1	0
<i>Digitalis purpurea</i> (foxglove)	3	120	136	103	0	0
<i>Fagopyrum esculentum</i> (buckwheat)	1	425	287	51	0	0
<i>Zinnia elegans</i> (zinnia)	3	184	98	2	0	0
<i>Linaria cymbalaria</i> (Kenilworth ivy)	2	224	252	89	0	0
<i>Tagetes patula</i> (French marigold)	2	208	163	108	0	0

The data presented in table 5, using the number of lesions on *Nicotiana glutinosa* as the criterion, suggest that in extracts of *Phacelia whittlavia* infected with tobacco mosaic the virus was, to some extent, inactivated at 85° C., and completely inactivated at 90° C. Similar results were obtained with foxglove, buckwheat, zinnia, Kenilworth ivy, and French marigold. The results secured with spinach, however, are distinctly different. With the nontreated extract, a small number of lesions were produced on *N. glutinosa* and a larger number were produced with extracts heated at 80° C. Inactivation of the virus was not obtained at 90° C., as was indicated for all the other hosts except tobacco. While the results obtained from control inoculations made with the various nontreated plant extracts show considerable variation in the number of lesions produced, it is difficult to attach much significance to these variations except in the case of such marked differences as occur in spinach. This exception will be considered in more detail later.

The data presented (Tables 4 and 5) support the generally accepted conclusion that the thermal death point of the tobacco-mosaic virus is close to 90° C. for a 10-minute treatment, regardless of the host in which it

develops. Although *Nicotiana glutinosa* is of value in detecting differences in relative concentration of the virus, it apparently is not so sensitive as *N. tabacum* in the detection of very low concentrations of virus.

*Tolerance to dilution.* The results secured by diluting the infectious extract from various species with different amounts of water followed by inoculation to tobacco are presented in table 6. A relatively high per-

TABLE 6.—Results of dilution trials with the tobacco-mosaic virus from different host species following inoculation to *Nicotiana tabacum*

Source of inoculum	Dilution of virus					
	None	1 to 100	1 to 1,000	1 to 10,000	1 to 100,000	1 to 1,000,000
<i>Nicotiana tabacum</i> (tobacco)	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{8}$
<i>Phacelia whittlavia</i> (California blue bell)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{11}$
<i>Spinacia oleracea</i> (spinach)	$\frac{25}{22}$	$\frac{25}{23}$	$\frac{25}{25}$	$\frac{25}{25}$	$\frac{25}{25}$	$\frac{25}{19}$
<i>Digitalis purpurea</i> (foxglove)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{9}$
<i>Fagopyrum esculentum</i> (buckwheat)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{11}$	$\frac{15}{6}$	$\frac{15}{5}$
<i>Zinnia elegans</i> (zinnia)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{14}$	$\frac{15}{7}$	$\frac{15}{1}$
<i>Linaria cymbalaria</i> (Kenilworth ivy)	$\frac{24}{22}$	$\frac{24}{20}$	$\frac{24}{16}$	$\frac{24}{15}$	$\frac{24}{11}$	$\frac{24}{6}$
<i>Tagetes patula</i> (French marigold)	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{7}$	$\frac{10}{6}$	$\frac{10}{3}$

centage of infection was secured on tobacco plants inoculated with *Phacelia whittlavia* extract diluted to 1 to 100,000 and 1 to 1,000,000. Similar results were obtained with the dilutions of extracts from infected spinach, foxglove, and tobacco. In the case of buckwheat and Kenilworth ivy only fair percentages, and with French marigold and zinnia low percentages of infection were secured at these higher dilutions. These differences may be explained partly on the basis of virus distribution and concentration in the various original extracts. It has been shown that the virus spreads erratically in French marigold, Kenilworth ivy, zinnia, and buckwheat, and it is possible that the original extracts from these hosts did not contain the virus in such highly concentrated condition as might be expected from tobacco or



*Phacelia whittlavia*. However, it is significant that some infection was obtained from all hosts used at the highest dilution of 1 to 1,000,000.

Inoculations of some of the above diluted extracts also were made to *Nicotiana glutinosa*, and the average number of lesions appearing on 5 leaves is recorded in table 7. With diluted extracts of *Phacelia whittlavia*,

TABLE 7.—Results of dilution trials with the tobacco-mosaic virus from different host species following inoculation to *Nicotiana glutinosa*

Source of inoculum	Number trials	Av. number of lesions on 5 leaves					
		Dilution of virus					
		None	1 to 100	1 to 1,000	1 to 10,000	1 to 100,000	1 to 1,000,000
<i>Nicotiana tabacum</i> (tobacco)	2	670	463	233	149	46	17
<i>Phacelia whittlavia</i> (California blue bell)	2	493	339	115	81	32	3
<i>Spinacia oleracea</i> (spinach)	5	16	22	46	67	13	12
<i>Digitalis purpurea</i> (foxglove)	2	237	162	134	113	22	3
<i>Fagopyrum esculentum</i> (buckwheat)	2	356	119	18	3	2	1
<i>Zinnia elegans</i> (zinnia)	2	166	41	11	2	1	1
<i>Linaria cymbalaria</i> (Kenilworth ivy)	2	150	169	84	71	2	1
<i>Tagetes patula</i> (French marigold)	2	240	134	56	41	15	1

the average number of lesions decreased as the amount of dilution increased. Similar results were obtained with diluted extracts from infected foxglove, buckwheat, zinnia, French marigold, and tobacco. These data support the conclusion that the number of lesions appearing on *N. glutinosa* may be used in a general way to indicate the relative amounts of virus present. However, there were apparently certain cases in which this was not true. The average number of local lesions appearing after inoculation with undiluted Kenilworth ivy extract was less than that obtained from extract diluted to 1 to 100. In this instance the results obtained were, as nearly as could be determined, due to the toxic effect of the Kenilworth ivy juice on *N. glutinosa*, as indicated by necrosis in the leaf areas inoculated. Similar toxic action resulted from extract from healthy Kenilworth ivy.

The toxicity of the juice of this plant was not always apparent, and it is believed that this toxicity may in some way be associated with environmental conditions existing at the time of or subsequent to inoculation.

The results obtained by inoculation with spinach extracts were strikingly different from those secured with extracts of other hosts. In the case of spinach extract the average number of lesions was always low, even though the undiluted extract was obtained from leaves showing good mottling. Similarly, fewer lesions were produced on *Nicotiana glutinosa* with the undiluted extract than at dilutions of 1 to 1,000 to 1 to 10,000. The apparently deleterious influence of spinach on the tobacco mosaic virus seemed to be of sufficient significance to warrant further study.

*Influence of plant extracts on the virus.* Allard (1), in 1914, carried out experiments to determine whether the sap of mosaic plants could be rendered innocuous by mixing it with different portions of the sap of what were believed to be immune species. He used extracts of healthy *Nicotiana viscosum* (*N. glutinosa*) and *N. glauca* and found that the treated virus in no instance lost its infectious properties. In 1925 Duggar and Armstrong (10) also studied the effect of treating the virus of tobacco mosaic with the juices of various plants. They found that the juice of pokeweed, *Phytolacca decandra*, effectively inactivated the agency of tobacco mosaic in a relatively short time (15 to 18 hours). Even when the virus was diluted only in a proportion of 1 part virus extract to 5 parts pokeweed juice, the inhibition was complete. Inactivation also was caused by the juice of Jimson weed, a solanaceous plant, where the latter was in relatively high concentration, and, to some extent, also, by the juice of geranium. On the other hand, cotton, squash, potato, sweet potato, and apple exerted no injurious influence at the concentrations tested. They state that it seemed conceivable that inactivation of the mosaic virus might be due to adsorption.

In the present investigation the healthy plants selected as sources of extracts for treatment of the tobacco-mosaic virus were chosen for various specific reasons. Spinach plants were used because of the unusual behavior noted in the previous tests (Tables 4, 5, 6, and 7). *Nicotiana glutinosa*, beet, Swiss chard, and bean were employed because of the fact that they exhibited only a localized type of infection when inoculated with the ordinary tobacco-mosaic virus. Snapdragon was used because of the low percentage of infection obtained (Table 2) from inoculation with the ordinary tobacco-mosaic virus. *Phytolacca rigida* Small, was chosen as a source of extract because of its close relationship to *P. decandra* which has previously been shown to have an inactivating or adsorptive effect upon the virus. Dilution with water and with extracts from healthy tobacco plants were made as controls.

The method employed was essentially as follows: leaves from healthy plants were ground in a meat chopper, the juices strained through cheese-

cloth, and 9-cc. portions placed in test tubes. The extract containing virus was obtained by crushing leaves of infected tobacco plants in a mortar and straining the juice through cheesecloth. A 1-cc. portion of this infective extract was placed in each test tube containing the 9 cc. of healthy plant juice. The test tubes were then shaken for about 2 minutes, and 2-cc. portions of the mixture were poured off and used to inoculate 5 leaves of *Nicotiana glutinosa* plants. The remaining 8 cc. of mixtures were placed in the ice box at 4° C. until the following day (18 to 24 hours later), when the tubes were removed, shaken, and a 2-cc. sample of each poured off and again inoculated to *N. glutinosa*. The number of local lesions appearing on 5 leaves after a period of 3 to 5 days was recorded, and the average number of lesions for all trials is shown in table 8. In each case a portion of the

TABLE 8.—The influence of various plant extracts on the tobacco-mosaic virus, as tested by inoculation to *Nicotiana glutinosa*

Source of healthy juice	Number trials	Av. number of lesions on 5 leaves	
		Time of inoculation	
		Immediately	After 1 day
<i>Nicotiana tabacum</i> (tobacco)	3	682	668
<i>Nicotiana glutinosa</i>	3	460	639
<i>Antirrhinum majus</i> (snapdragon)	3	322	388
<i>Phaseolus vulgaris</i> (bean)	3	232	224
<i>Spinacia oleracea</i> (spinach)	3	16	12
<i>Beta vulgaris</i> (beet)	3	32	26
<i>Beta vulgaris</i> var. <i>ciela</i> (Swiss chard)	3	9	11
<i>Phytolacca rigida</i>	3	10	5
Water control	3	724	750

extracts of the healthy plants also was inoculated to *N. glutinosa* as an added check on the absence of the virus from the plants. In all tests of these extracts no local lesions were obtained. Furthermore, these juices caused no macroscopically visible toxic effects on the inoculated *N. glutinosa* leaves.

One-cc. portions of ordinary tobacco-mosaic-virus extract from tobacco

diluted with 9 cc. of distilled water produced about 700 local lesions on *Nicotiana glutinosa* (Table 8). Similar results were secured with dilutions of virus with extracts of healthy tobacco plants. The average number of lesions obtained from dilutions with extracts of *N. glutinosa*, snapdragon, and bean was somewhat lower. The number secured from dilutions with the extracts of spinach, beet, Swiss chard, and *Phytolacca rigida* was significantly lower. It is also interesting to note that the results obtained from inoculations made immediately after mixing and those made one day later were not essentially different. In any one series of tests the amount of virus present in each 1-cc. portion should be approximately the same because the portions were obtained from a single sample of juice. The average of the 3 trials as presented in table 8 is representative of the results obtained in each series of tests.

It is apparent from the above results that extracts from beet, Swiss chard, spinach, and *Phytolacca rigida* have a deleterious effect on the infectiousness of the ordinary tobacco-mosaic virus. The low number of local lesions obtained on *Nicotiana glutinosa* with extracts from infected spinach (Tables 4, 5, 6, and 7) may not, therefore, be attributable merely to a low concentration of the virus in this nonsolanaceous host.

*Resistance to Aging in Vitro.* Extracts of tobacco containing the ordinary tobacco-mosaic virus are known to retain their infectious nature for long periods of time. It was thought desirable to determine whether the length of time that the virus could resist aging would be materially reduced in extracts obtained from infected nonsolanaceous hosts. The 8 species selected for sources of infectious extract were: California bluebell, *Phacelia whittlavia*, *P. grandiflora*, *P. parryi*, Kenilworth ivy, foxglove, spinach, tassel flower, and Chinese forget-me-not. Extract from an infected tobacco plant was used as a control. The aging test was made by placing 10 cc. of the extracts in stoppered test tubes for a period of 3 months in a darkened drawer in a greenhouse where the temperature usually ranged between 80 to 90° F. Following this treatment, inoculations were made to tobacco plants to determine the presence of the virus. All the extracts employed in this test were found to have retained their infectious properties for the 3-month period.

#### Host Influence on Virus Attenuation

In 1925 Carsner (5) found that passage of the virus of curly-top of sugar beet through certain resistant plants resulted in attenuation of the virus. Recently, Lackey (27) reports that attenuated virus of sugar beet curly-top, obtained by passage through *Chenopodium murale* L., was restored to its original virulence by passage through *Stellaria media* (L.) Cyr. Johnson and his coworkers (22, 24) have shown that several different

environmental factors may bring about attenuation of the tobacco-mosaic virus. They found that virulence of this virus, as measured by symptom expression, was not restored by repeated transfer through tobacco plants. A case of attenuation of the tobacco-mosaic virus as a result of passage through *Martynia* also has been reported from this laboratory (25).

These instances of attenuation suggested the desirability of testing the effect of other nonsolanaceous hosts on the tobacco-mosaic virus. The 5 species selected for study were: *Phacelia whittlavia*, larkspur, buckwheat, tassel flower, and French marigold. Successive transfers of the ordinary tobacco-mosaic virus were made through a series of young plants of each species. With each transfer of a portion of the infectious extract to the respective species, inoculations were also made to tobacco for the purpose of noting possible modification of symptoms. At the same time inoculations were made to *Nicotiana glutinosa* for the purpose of readily checking on the specificity and the amount of virus transferred in each case. Controls were simultaneously inoculated with virus extract obtained from tobacco.

In this manner the virus was transferred through 4 successive series of *Phacelia whittlavia*, larkspur, buckwheat, tassel flower, and through 3 series of French marigold plants. No definite evidence of attenuation was secured in these trials. On the basis of these preliminary tests it is concluded that the ordinary tobacco-mosaic virus is at least not regularly nor readily attenuated by passage through nonsolanaceous host plants.

### Cytological Observations

Microscopic examinations of the tissues of tobacco plants infected with the ordinary tobacco-mosaic virus have been made by numerous investigators. Two types of cell inclusions, namely, x-bodies and striate material, have been described in association with tobacco mosaic on tobacco. In 1927, Hoggan (14) found these 2 types of cell inclusions to be a constant feature of the ordinary tobacco-mosaic virus on other solanaceous host plants that developed definite symptoms of mottling or chlorosis. In 1931, Hoggan (15) transmitted the ordinary tobacco-mosaic virus to *Martynia louisiana* and reported the presence of characteristic cell inclusions in this host also.

The method employed in the present investigation was essentially the same as that described by Hoggan (14). Small portions of healthy and diseased plant tissues were cut out of the leaf lamina with a sterile safety-razor blade, placed immediately in the fixing fluid (100 parts 50 per cent alcohol, 6.5 parts Formalin, and 2.5 parts glacial acetic acid), and allowed to remain 24 to 48 hours. The usual procedure for embedding in paraffin was carried out. The material was sectioned at a thickness of  $7\mu$  in a

plane perpendicular to the leaf surface, and stained with Heidenhain's iron-alum haematoxylin.

The stained sections of infected tissue from chlorotic leaves of *Phacelia whittlavia* showed the presence of numerous x-bodies and some striate material, (Fig. 3, A, B). Bodies and material of similar form and size were found in chlorotic tissue of larkspur (Fig. 3, D), Kenilworth ivy, and foxglove. In spinach (Fig. 3, C), however, a lesser number of x-bodies and

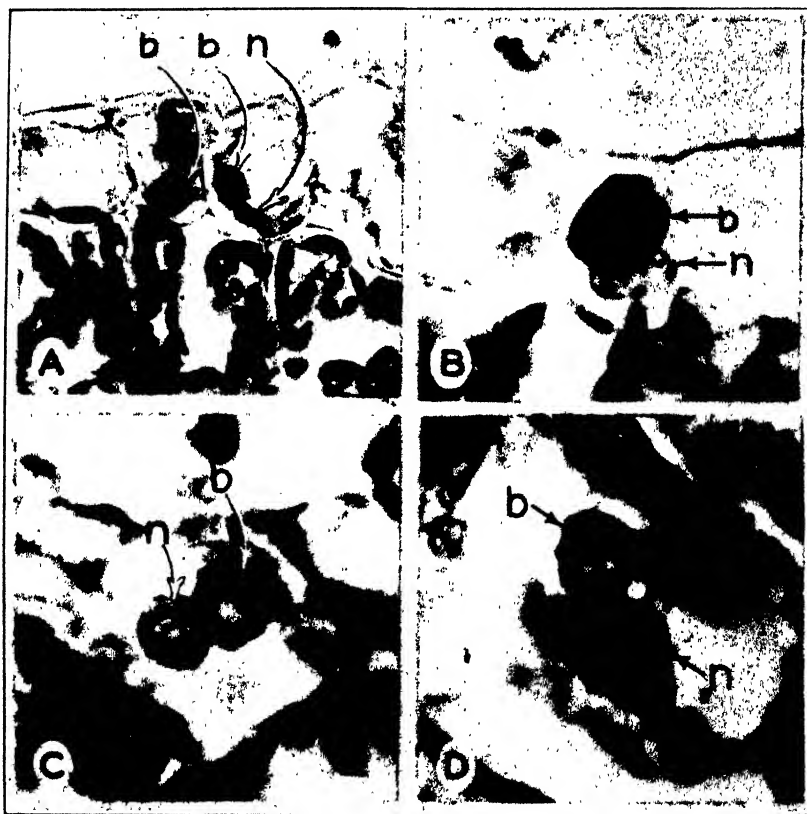


FIG. 3. Photomicrographs showing x-bodies (b), and nucleus (n), in leaf tissue of nonsolanaceous hosts infected with tobacco mosaic. A. *Phacelia whittlavia* (California blue bell).  $\times 700$ . B. *P. whittlavia*.  $\times 1,600$ . C. *Spinacia oleracea* (spinach).  $\times 1,750$ . D. *Delphinium consolida* (larkspur).  $\times 1,600$ .

smaller amounts of striate material were observed, while in Maryland figwort larger quantities of striate material and only a few x-bodies were found. Examination of stained sections of comparable healthy tissues failed to show the presence of any such inclusions.

Observations of infected leaf tissue from buckwheat did not disclose

any typical inclusions, possibly because of the limited amount of material examined.

#### DISCUSSION

Casual observation of plants in nature and in culture, as well as frequent reports in literature, testify to the common occurrence of viruses in the flowering plants. The greater interest now lies in the particular virus concerned in each case, rather than in the occurrence of the disease itself. The number of different viruses which exist in nature may not be large as compared with other groups of parasites, and it is likely that they will eventually be fairly well classified. A desirable preliminary to any classification scheme for plant viruses is a general understanding of the host range of each specific virus. With this information as a basis, the utilization of differential hosts should be simplified and methods of determination shortened.

Only a few viruses have previously been intensively studied from the point of view of host range. The outstanding examples are the viruses of aster yellows (26), tobacco ring-spot (37) and curly-top of sugar beet (4, 34, 35). These viruses have very extensive host ranges, whereas other viruses, though not intensively investigated (*e.g.*, mild mosaic and crinkle mosaic of potato), evidently are very limited in host range.

The experimental data presented in this paper will aid in clearing up some obscure conceptions relative to the classical tobacco-mosaic virus. The theoretical host range of this virus has passed through a succession of stages. While it was at first conceded only a very limited host range, it was later associated in a confusing manner with other viruses, which, as a group, were reported to be intertransmissible among species that are widely separated taxonomically (12). For a time these results were interpreted by some as applying to the ordinary tobacco-mosaic virus, whereas this virus was apparently only one of several that were being employed without distinction.

With the more general recognition of virus specificity, the host range was then believed to be limited to the Solanaceae (21). The present investigations show, however, that it may be transmitted experimentally to other widely separated families among dicotyledonous plants. Furthermore, the high percentage of infection secured (24 per cent) among the species tested suggests that still other species in other families will, when tested, prove susceptible.

On the whole, the 29 nonsolanaceous host species reported in this paper are not highly susceptible, but the variation in behavior in this respect is apparently not much greater than that to be found in the solanaceous family itself. The range of symptom expression in the susceptible non-

solanaceous species is also quite comparable to that described by Holmes (18) within the Solanaceae.

The behavior of the virus of ordinary tobacco mosaic with respect to its properties and the occurrence of the characteristic cell inclusions in several of the nonsolanaceous hosts add support to accumulating data on the specificity and stability of this virus under varying conditions. Further evidence of the stability of this virus is shown by the failure to obtain attenuation by passage through several series of widely separated nonsolanaceous hosts. The deleterious effect of the extracts of certain nonsolanaceous plants is not unusual, since a similar effect may be exercised by members of the Solanaceae, as in the case of *Datura stramonium* L. (10).

The results secured in this investigation also may eventually have some bearing upon the development of control measures for the disease caused by this virus. If wild or overwintering hosts should be found to play an important part in the origin and dissemination of the disease in local areas, it is evident that certain nonsolanaceous as well as solanaceous plants must be considered as probable "carriers."

#### SUMMARY

The host range of the ordinary tobacco-mosaic virus, generally regarded as limited to certain species within the family Solanaceae, has been extended to include 29 species, representing 14 widely separated families.

Symptom expression in these nonsolanaceous plants is characterized by marked variations ranging from masked symptoms to typical chlorosis, mottling, malformation, necrosis, and stunting, alone or in combination. The distribution of the virus in these plants varies from typical systemic infection to localized infection or erratic spread from the inoculated portion.

The host species in which the virus developed did not radically influence the constancy of the properties of the virus as measured by thermal death point, tolerance to dilution, and aging *in vitro*.

The virus did not become attenuated by passage through several series of certain nonsolanaceous hosts.

In the case of spinach infected with tobacco mosaic, the virus appeared to exist in only very low concentration. Extract from healthy spinach plants, however, acted deleteriously on virus extracts from tobacco of high concentration. Consequently, low percentages of infection secured from certain susceptible species are evidently not necessarily a result of low concentrations of the virus in such hosts.

The presence in several nonsolanaceous hosts of intracellular bodies commonly associated with the ordinary tobacco-mosaic virus has been established cytologically.

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# DOWNY MILDEW OF TOBACCO<sup>1</sup>

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## INTRODUCTION

Downy mildew of tobacco, commonly known as blue mold, occurs ubiquitously throughout the entire area devoted to the culture of flue-cured tobacco. Observations of the past 3 seasons indicate that it is primarily a disease of seedlings. They indicate also that the disease will continue to appear in epiphytotic proportions every spring, although it may vary in destructiveness.

Nearly all investigations of downy mildew that have hitherto been made, have been conducted in Australia where this disease is endemic. The results of these investigations, in their most essential features, are summarized in a recent publication by Angell and Hill (2). The present studies deal with such matters as the life history and morphology of the pathogen, the source of inoculum in spring, the agencies of dissemination, the pathology of the disease, and the relation of weather conditions to development and spread. They are, in part, confirmatory of previous investigations in Australia.

## HISTORY OF THE DISEASE

Definite records of downy mildew on tobacco in Australia date from 1890, although it was probably present 30 or 40 years earlier (2). The first record of its occurrence within the United States is that by Farlow (9) who noted it on *Nicotiana glauca*, an introduced ornamental species, near San Diego, California, in 1885. He prophetically stated at that time that, "It is much to be feared that the disease which attacks *Nicotiana glauca* may sooner or later extend to cultivated tobacco. If this happens the injury to the tobacco will be very great . . . . If *N. glauca* and its parasite are once introduced into the Gulf States, the parasite might attack the tobacco grown there and then pass on to Virginia and the other states where tobacco is the most important crop." Partial fulfillment of this prophecy came in March, 1921, when the disease made its appearance in Gadsden County, Florida, and Decatur County, Georgia, on seedlings of shade-grown tobacco. In their account of this outbreak, Smith and McKenney (13) pointed out

<sup>1</sup> A cooperative investigation by the Departments of Botany and Chemistry, Duke University. The writers wish to express their gratitude for their cooperation to Dr. P. M. Gross and Mr. E. G. Moss, and to the several farm demonstration agents and interested growers for their courtesies.

that collections of mildew on cultivated tobacco seedlings had been made in Texas, in 1906, and that the disease may have been introduced into the Florida-Georgia area by way of California and Texas. The possible spread to Florida through gradual extension of its range is indicated by the collection of downy mildew on *Nicotiana bigelovii* in Nevada (15). Burger and Parham (7) expressed the opinion that downy mildew probably existed in Florida over a period of years prior to 1921. Smith and McKenney (13) also suggested the possibility of its introduction with mats from the East Indies. Palm (11) maintained that this source of introduction is improbable because (1) the mats are made in Borneo, in a section where tobacco is not grown, (2) the causal organism is not known to occur in the Dutch East Indies, and (3) the chance of contamination, en route, by contact of mats with tobacco from China and India, or in storage in Holland with tobacco from Brazil and Europe, is remote.

For some unknown reason, downy mildew did not again appear in epiphytotic proportions until the early spring of 1931, when it occurred throughout Florida, Georgia, and Louisiana. Before the end of the season for transplanting it had appeared in many sections of South Carolina, North Carolina, and Virginia. In 1932 it appeared on seedlings in these last-named states considerably earlier in the season, and spread to all tobacco seed beds throughout the entire Coastal Plain and Piedmont sections of the Atlantic seaboard, as far northward as Maryland. In 1933, the disease spread to the Burley-tobacco sections of Western North Carolina, and of Tennessee, and extended its range as far northward as Pennsylvania.

#### SUSCEPTS

McAlpine (10), in early investigations of downy mildew in Australia, reported that the same organism that attacks cultivated tobacco occurs also on *Nicotiana suaveolens*, a species native to Australia. Adam<sup>2</sup> noted that *N. glauca*, *N. rustica*, and *N. longiflora* are also susceptibles. Angell and Hill (2) are of the opinion that all species of *Nicotiana* are probably subject to the disease because of their observations that seedlings of the following species are readily attacked: *N. angustifolia*, *N. atropurpureum*, *N. californica*, *N. paniculata*, *N. campanulata*, *N. sylvestris*, *N. nudicaulis*, *N. repanda*, *N. caudigera*, *N. triangophylla*, *N. chinensis*, *N. glutinosa*, *N. acuminata*, *N. langesdorfii*, *N. laternia*, and *N. bigelovii*.

The writers' observations indicate that seedlings of all agronomic varieties of flue-cured tobacco are equally susceptible to downy mildew. In addition, they have observed the disease on seedlings of tomato, *Lycopersicon esculentum*, pepper, *Capsicum annuum*, and eggplant, *Solanum melongena*, when these species were being grown near tobacco seed beds

<sup>2</sup> Reported from Adam's unpublished notes by Angell and Hill (2).

or in the same seed beds with diseased tobacco seedlings. Similar observations, recorded in the *Plant Disease Reporter*<sup>3</sup> have been made by a number of investigators. Potted seedlings of these three solanaceous species, when inoculated with sporangia from diseased tobacco seedlings, become infected about a week after inoculation.

*Hyoscyamus niger*, when grown in beds among diseased tobacco seedlings, or when inoculated in pots under conditions favorable for infection of tobacco seedlings, remains free from attack. These observations accord with those of Angell and Hill (2). Potato, *Solanum tuberosum*, remained free from attack when artificially inoculated. *S. nigrum* and *S. carolinense*, two weeds of rather common occurrence in or near tobacco seed beds, are also apparently immune from downy mildew. Other immune solanaceous species include Jerusalem cherry, *S. pseudo-capsicum*, and apple of Sodom, *S. sodomacum*, (Darnell-Smith (8)).

#### APPEARANCE OF THE DISEASE

Downy mildew is primarily a seedling disease in the flue-cured tobacco-growing area. It usually appears about 2 weeks prior to the time that the seedlings are of size suitable for transplanting. It may, however, attack seedlings of any age, even those just emerging, observations that confirm those of Angell and Hill (2).

The symptoms of the disease, its rate of spread throughout the bed, and the severity of attack, are modified by such factors as weather conditions, age and vigor of the seedlings, location of beds with reference to exposure and soil and air drainage, density of the stand, and construction and management of the beds.

The most characteristic feature of the downy mildew is the presence of a dense, downy coating on the underneath portions of mature lesions on diseased leaves (Fig. 2). The presence of the disease is first indicated by the general aspect of beds. One may note, on observing a bed in which the disease is first beginning to appear, that small groups or patches of seedlings are yellowish green in color. There may be only one such patch or at most a few, even in beds several hundreds yards in area. These areas may appear in any portion of the seed bed but are most commonly present around stumps or in places in which the seedlings are making a subnormal growth. Closer inspection of yellowish green seedlings reveals that the tips of many of the upper leaves droop and are flaccid. At this stage the sporangia and sporangiophores of the causal fungus may not yet have appeared. The under surface of the lower leaves of such plants, however, may be partly or entirely covered with a downy felt. During periods favor-

<sup>3</sup> U. S. Dep. Agr., Bur. Pl. Indus. Pl. Dis. Rptr. 17: 37-39. 1933.

able for the development of the pathogen, as during cool weather in which the sky is continuously overcast, the downy coating may appear on the upper leaf surface, also. This is of common occurrence on leaves in thickly sown beds or on the most shaded leaves. When, at this time, affected leaves are viewed from above, they show large, irregular, yellow patches or the leaves may be uniformly yellowish (Fig. 1). Large, dry, irregular areas



FIG. 1. Downy mildew on upper and lower surfaces of leaves of tobacco seedlings.

replace the yellow patches within a few hours on clear windy days. Under these conditions the general aspect of the seed bed is as of plants that have been scalded. This scalding is most pronounced when clear weather follows a cloudy period during which every seedling has become involved. The rapid collapse of seedlings is accompanied by a characteristic vile odor, quite like that of decaying cabbage.

Usually, small seedlings are killed outright, but the "bud leaves" of the larger ones remain apparently normal, even though all the lower leaves have collapsed. Such plants as survive may have recovered sufficiently within 2 weeks to be transplanted.

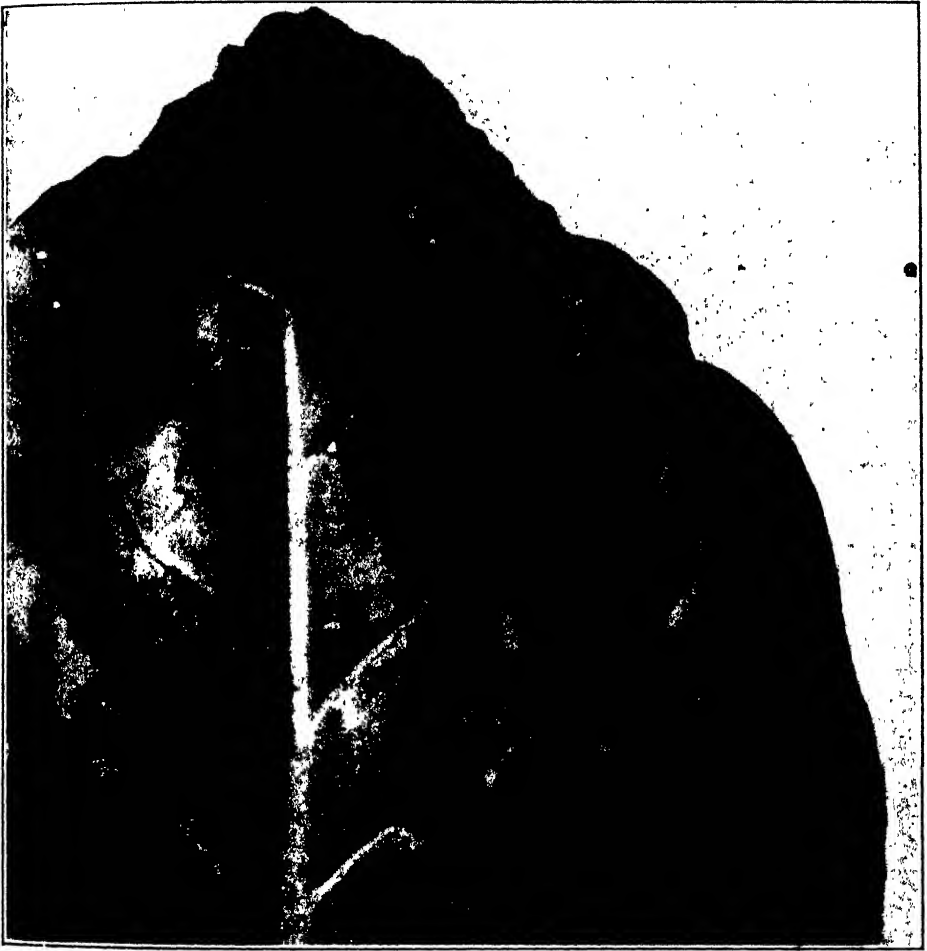


FIG. 2. Lower surface of lesion, enlarged, showing a profuse coating of the sporangial stage of *Peronospora nicotianae*.

Pittman (12) regards the name "blue mould" as inappropriate because the least conspicuous feature of the fungus is its blueness. When, however, fresh material is examined in early morning or when there has been a period characterized by rainy days with cold nights, the down has a pronounced violet tint. When the lesions have dried or after several hours of sunshine the fructifications, *en masse*, are grayish to pale brown.

If, after a brief period favorable for infection, a period of dry weather prevails, indefinite yellow specks appear on the upper leaf surface. These are abortive lesions, which may persist unchanged or their centers may become necrotic. The growth of the normal tissue surrounding these abortive lesions may result in the leaf surface becoming puckered or contoured. Such lesions may never bear a crop of sporangia if the weather remains dry and warm. It is for this reason that there seems to be no way of determining in advance, from the appearance of any given infected leaf, whether sporangia will appear and of prophesying the date of such an appearance.

During a period favorable for the development of the disease the collapse of infected leaves usually follows within a few hours the production of sporangia on those leaves. In some cases, though, daily crops of sporangia may be formed along the advancing margin of a given lesion.

Seedlings that have survived an attack of downy mildew and have developed new leaves, seen not to be subject to a second attack. This observation is supported by numerous field observations and by attempts to artificially inoculate seedlings that have recovered. No satisfactory explanation for the apparent immunity can be made at this time.

Downy mildew occurs in Australia on the leaves of mature plants and causes the formation of large lesions that may ultimately drop out(2). It may also appear there, during autumn, on the suckers, flower buds, flowers, and capsules. It is improbable, because of the relation of weather conditions to the development of the causal fungus, as will be pointed out later in this report, that downy mildew will attack the growing crop in the field in the flue-cured area.

#### THE CAUSAL ORGANISM

*Morphology.* The fungus that causes downy mildew of tobacco has, in common with many other downy mildews, two life-cycle stages, *i.e.*, sporangia and oospores. The sporangia are borne at the surface of the lesions prior to the death of the tissues and the oospores, within the dead leaf tissues.

The sporangia are elliptical to oval bodies 15–28 by 12–18  $\mu$ , and are densely filled with pigmented, granular material and scattered oil droplets. They are borne on dendritic, dichotomously branched sporangiophores, which branch 4 to 8 times, and terminate in curved, acute apices (Fig. 3, A). The sporangiophores occur either singly or in small groups. They range in height from 400 to 750  $\mu$ . The diameter of their bases is 10 to 12  $\mu$ . The length of the stalk approximates two-thirds the height of the sporangiophore.



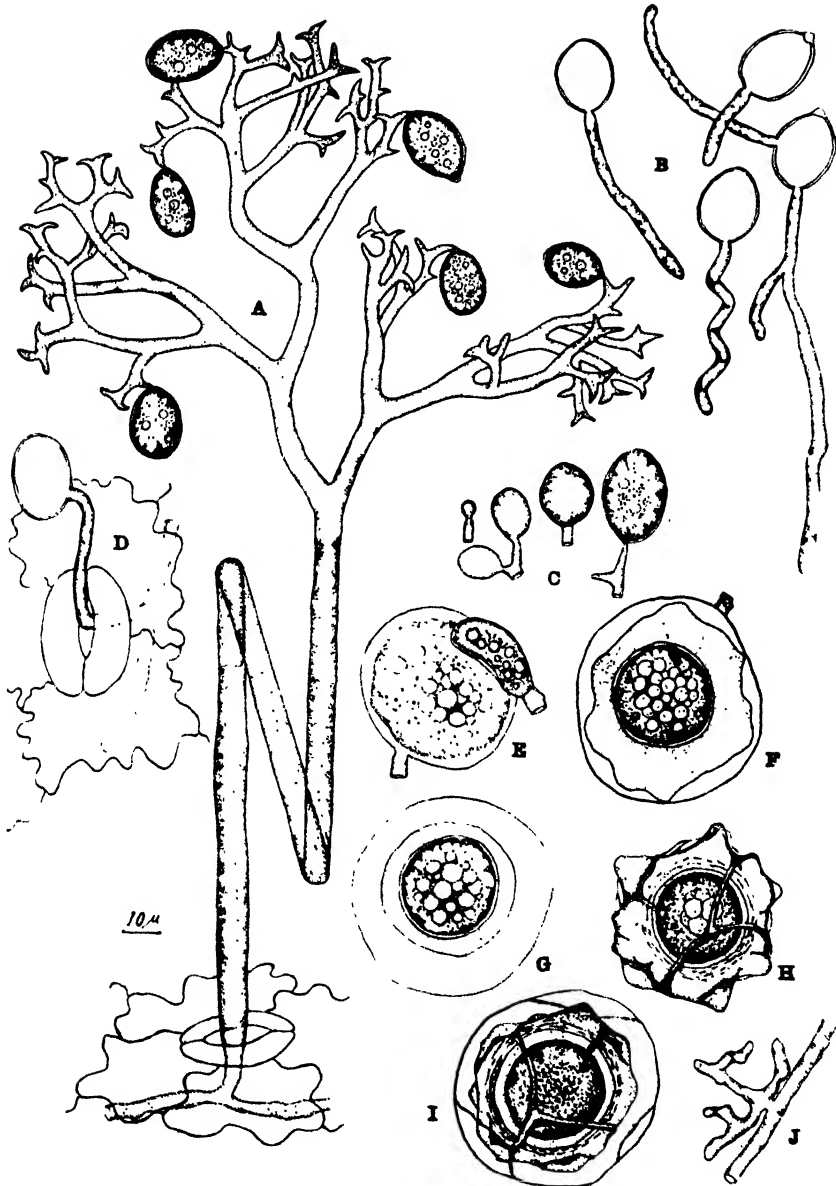


FIG. 3. A. Sporangiphore of *Peronospora nicotianae* from which most of the sporangia have been dislodged. B. Germinating sporangia. C. Stages in the matinal development of sporangia at the apices of sporangiphore. D. Infection tube entering a stoma. E. Young spherical oogonium with a clavate antheridium applied to it. F. and G. Stages in the development of the oogonium, in which the hyaline tunic and the brown periplasm and oospore have been differentiated. H. and I. Mature oospores with thickened wall. J. Haustorium.

Sporangial formation has been studied by stripping off the epidermis of infected leaves and examining under a microscope. Sporangiphores arise as branches from the intercellular hyphae and emerge from the stomates. They soon become bifurcately branched, and, as development continues, a dendritic structure with blunt, dichotomous tips arises. These enlarge into bulbous processes, (Fig. 3, C), and by the time they have attained approximately half their ultimate size, a septum delimits the young sporangium from the sporangiphore. At this stage the fructifications are colorless, but, because they cover the lesions so copiously, the leaf surface appears to be covered with a white down. By the time the sporangia have attained their mature size, their color is distinctly violet, and the sporangiphore tips have become curved and taper-pointed.

As the result of a series of all-night vigils it was found that sporangial production is matinal. It begins with the advent of dawn and the sporangia are mature by sunrise. If the weather is cloudy maturity is somewhat delayed. Conceivably, this process could continue throughout dark days, but this is not known to be true.

Mature oospores have been observed in material collected from such widely separated localities as Lumberton, Wilson, Townsville, Oxford, and Apex, North Carolina, and Blackstone, Virginia. They occur within the tissues of old leaves that have collapsed and remained in contact with the soil, also in leaves kept in moist chambers 4 or 5 days after the tissues have become necrotic. When such tissue is examined with low-power magnification, the oospores appear as bright, reddish brown spheres embedded within the decaying leaf, and may be so abundant that 100 or more can be seen in a microscopic field.

Young oogonia are spherical structures, hyaline, but filled with an abundance of refractive granules and oil globules (Fig. 3, E). The antheridium is a bluntly clavate structure, somewhat concave on the surface applied to the oogonium (Fig. 3, E). Apparently, coincident with fertilization, the ooplasm becomes differentiated into a thin-walled, dense, pale brown, central oosphere with a less dense periplasm and an outer, hyaline envelope (Fig. 3, F and G). As the oospore matures, its wall thickens and the depth of color increases until it finally becomes reddish brown. Meanwhile, the periplasm becomes contoured into low, blunt elevations and ridges (Fig. 3, H and I). The hyaline, outer tunic collapses with desiccation and resumes its normal form when moistened. Oogonia range from 60 to 85  $\mu$  in diameter and the oospores from 45 to 75  $\mu$ . It has not been possible to accomplish the germination of these oospores.

*Identity.* The organism that causes downy mildew of tobacco has hitherto been designated *Peronospora hyoscyami* deBary (6, p. 123-124), an organism described in 1863, on *Hyoscyamus niger*. It would appear

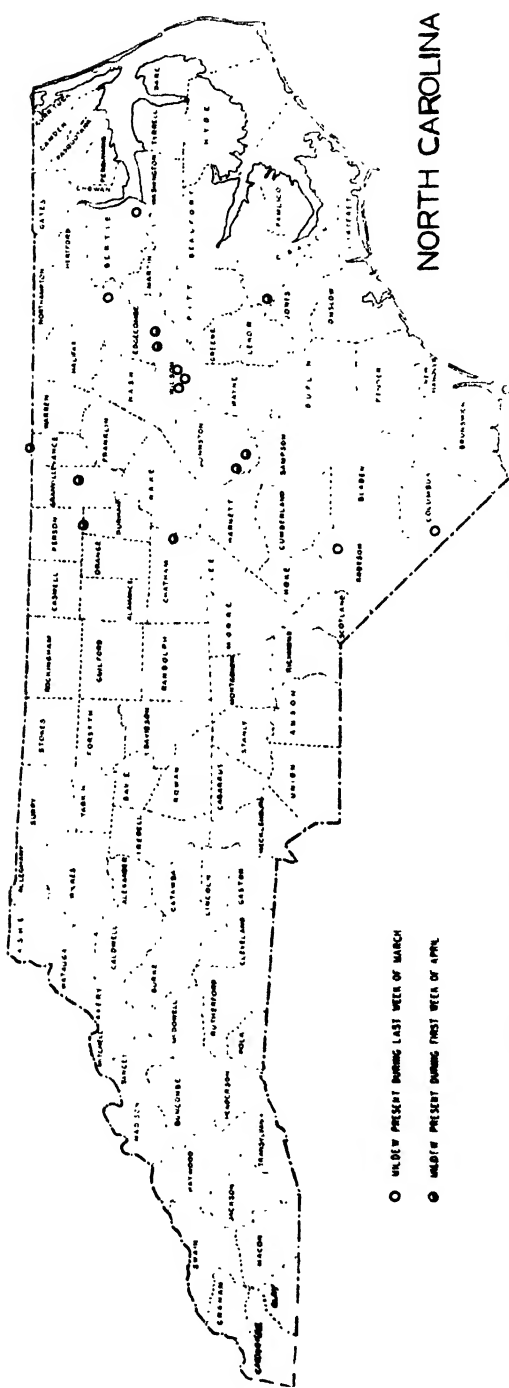


FIG. 4. Loci of early outbreaks of downy mildew, originating from seed beds located on old sites.

from the observations of Angell and Hill (2), and the previously mentioned inoculation experiments of the writers, that *H. niger* is immune from the pathogen that causes downy mildew of tobacco. Since downy mildews are all obligate parasites that are rather restricted in suscept range, it seems more reasonable, therefore, to regard the downy mildew of tobacco as specifically distinct from *P. hyoscyami*. It does not appear probable that immunity of *Hyoscyamus niger* from the tobacco fungus can be explained by physiological modification or changed virulence of the pathogen.

In 1891 Spegazzini (14, p. 36) described *Peronospora nicotianae* on *Nicotiana longiflora* from collections made in Argentina. Unfortunately, Spegazzini's type specimens are not available for comparison. The variability of the downy mildew on cultivated tobacco suggests that a direct comparison of it with type specimens of *P. nicotianae* might still leave a doubt as to their identity. This variation is shown in the summary, which follows. Angell and Hill (2) pointed out that the maximum, minimum, and average measurements of sporangia for the organism in Australia are each 50 per cent greater in January than in May. Their length measurements lay between 12 and 34  $\mu$ , and the breadth between 10 and 20  $\mu$ , with 22 by 16  $\mu$  as the average dimensions. Spegazzini's measurements are 18–20 by 9–12  $\mu$ , and ours are 15–28 by 12–18  $\mu$ .

There is, in Australia, also a seasonal variation in size of sporangiophores. Angell and Hill's (2) measurements, in the growing season, show a range in height of from 589 to 1024  $\mu$ , in spread of crown of from 230 to 435  $\mu$  and in number of times the sporangiophores branch of from 5 to 8; in winter the height is 243  $\mu$ , the breadth 89  $\mu$ , and the number of times branched, 4. Spegazzini's height measurement is 250 to 500  $\mu$ , and ours 400 to 750  $\mu$ .

Angell and Hill (2) state that oospores are rarely found in Australia and give 28 to 50  $\mu$  as the range in size; the size recorded by Spegazzini is 50 to 80  $\mu$  and ours 45 to 75  $\mu$ .

In determining the identity of the downy mildew of cultivated tobacco, consideration must also be given to the fact of its ability to parasitize numerous species of *Nicotiana*, including *N. longiflora*, and certain other solanaceous species. This points to the conclusion that it is identical with *P. nicotianae* or else that there are 2 species of *Peronospora* on *Nicotiana*. In the case of the latter alternative, the downy mildew of cultivated tobacco is to be regarded as an undescribed species. It seems preferable, however, in the light of the facts that have been recounted, to regard the pathogen on cultivated tobacco as identical with *P. nicotianae*.

#### PATHOLOGY OF DOWNY MILDEW

In studying the relation of the organism to invaded tissues and to the changes that the fungus produces in them use was made of stained, paraffin

sections and of preparations made by stripping off the epidermis. Penetration is accomplished by entrance of the infection hypha through the stomates, (Fig. 3, D), as reported by Darnell-Smith (8). The coarse, coenocytic mycelium that arises passes between the cells and, thence, throughout the leaf tissue. Branched haustoria, (Fig. 3, J), are formed, which penetrate the cell walls. This is best demonstrated in epidermal strippings. Four to 7 days after inoculation, sporangiophores are produced. With the production of sporangia the cells die and rapidly collapse. This collapse is not confined to the tissues actually invaded but may involve the entire leaf or entire plants. This effect on uninvaded tissues is borne out by the experience of many growers who transplanted diseased seedlings or apparently quite normal plants from seed beds in which the disease was rapidly developing. When this was done, both in 1932 and 1933, usually 50 to 90 per cent of the seedlings failed to survive transplantation unless transplanting was delayed until the seedlings had apparently recovered. It appeared that the actual loss of leaf surface, resultant from leaf lesions was insufficient to account for this high mortality in transplanting diseased seedlings. For this reason attempts were made to determine if toxic products are produced within diseased tissues. Accordingly, a quantity of fresh leaves of diseased seedlings were macerated by grinding in a meat chopper. The crushed material was then covered with distilled water and allowed to extract for 15 hours at 38° F. The liquid portion was then removed by filtration through filter paper and placed in 5 small flasks. Four healthy tobacco seedlings were then removed intact from the soil and placed, with their root systems immersed, in the filtrate. The filtrate in the fifth flask was heated to boiling before placing a seedling in it. A filtrate from healthy tobacco seedlings was similarly prepared and 2 seedlings were placed in it to serve as a control. After 20 hours the 5 plants in the extract from seedlings affected with downy mildew were severely wilted, whereas those in the filtrate from healthy seedlings were turgid. The roots of the wilted plants were then rinsed and allowed to remain in tap water to permit recovery of turgidity. No recovery resulted, however.

In a comparable test, dead dried leaves from diseased seedlings, instead of fresh ones, were extracted in distilled water over night, at 38° F. This extract also caused healthy seedlings, whose roots were immersed in it, to wither. An extract, similarly prepared, from apparently normal leaves of seedlings just beginning to recover from downy mildew, also caused healthy seedlings to wither when their roots were immersed in it. In this case, however, the wilting was less pronounced and was most noticeable on the lower leaves. These tests are interpreted to show that toxic water-soluble substances are formed within diseased tissues and that they may permeate

the entire plant. These toxic substances may account, in part, for the death of affected seedlings following transplantation.

#### DISSEMINATION OF SPORANGIA

There are several agencies concerned in the dissemination of sporangia of downy mildew of tobacco. The spread of the disease either within an affected seed bed or from a diseased seed bed to a near-by healthy one could be readily accomplished by carriage of sporangia on the hands and clothing of laborers, (2), (13). It also has been demonstrated that, in Australia, sporangia are transported by moths of the split form, *Phthorimaea operculella* Z. (5).

The fact that the sporangia are powdery and so readily dislodged suggests that air currents should constitute the most important agency for their dissemination, as indicated in previous investigations (2), (13). Direct evidence of the transport of sporangia by air currents was obtained by means of spore-traps. These traps consisted of glass plates, 3½ by 4½ inches, covered with a film of glycerine and exposed, usually to leeward, in the vicinity of diseased seed beds. After exposure, the film of glycerine and adhering material were washed off, the washings were then centrifuged, and the sediment was examined with a microscope.

It was also found that sporangia are entrapped by glandular hairs on tobacco leaves, on the corollas of *Azalea nudiflora* or on the leaves of *Liquidambar styraciflua*, and by the resinous young shoots of pines. They could be washed off from these plant materials and collected in the sediment in centrifuge tubes. Some difficulty was experienced in identifying the sporangia of *Peronospora nicotianae* because 3 other morphologically distinct downy mildews were more or less prevalent; namely, *P. parasitica* (Pers.) deBy., on cruciferous weeds, *P. viciae* (Berk.) deBy., on *Vicia angustifolia* (L.) Reichard, and *P. geranii* Pk., on *Geranium carolinianum* L.

The results of studies on trapping sporangia of the downy mildew of tobacco are summarized in table 1.

It may be noted, first of all, that sporangia occurred on most of the glycerine slides and on most of the plant material. In some cases a single sporangium or at most a few were found in the sediment; in others sporangia were abundant. It is conceivable that the number of sporangia in the air would be governed by such factors as distance from infected beds and the wind velocity. Diseased beds from which these sporangia were liberated varied from a few feet to a few miles. The wind velocity at the time when the sporangia were entrapped varied from 200 to 400 feet per minute.

When it was determined that sporangia are air-borne, an effort was made to utilize this fact in predicting the occurrence of an outbreak at a

TABLE 1.—Dissemination of downy mildew by air currents

Date	Locality	Material examined	Results
4-13-33	Wilson, N. C.	Glycerine slide exposed at edge of diseased seed bed	Sporangia found
"	"	Glycerine slide exposed 25 ft. from diseased bed	No sporangia found
"	"	Glycerine slide exposed inside diseased bed	Sporangia found
"	"	Leaves of tobacco seedlings from bed 1000 ft. distant from diseased bed	" "
4-14-33	"	Leaves of <i>Liquidambar styraciflua</i> near diseased bed	" "
"	"	Glycerine slide exposed 1000 ft. distant from diseased bed	No sporangia found
4-15-33	"	Leaves of tobacco collected 1000 feet distant from diseased bed	Sporangia found
"	"	Flowers of <i>Azalea nudiflora</i> 300 ft. distant from diseased bed	" "
"	"	New shoot of pine collected near diseased bed	" "
4-17-33	"	Leaves of tobacco seedling collected at 8:30 A. M. 6 hrs. after rain, at a point 50 ft. distant from diseased bed	" "
"	"	Leaves of tobacco from healthy bed 1000 ft. distant from diseased bed	" "
"	"	Flowers of <i>Azalea nudiflora</i> near diseased bed	" "
"	"	Glycerine slides (5) exposed 20, 100, 225, 300 and 600 ft. to leeward of diseased bed	" "
4-23-33	"	Tobacco leaves from plant bed that showed no evidence of infection	No sporangia found
"	"	Tobacco leaves from plant bed that showed no evidence of infection	" " "
"	"	Glycerine plate exposed for a few minutes near slightly infected bed	" " "
"	"	Glycerine plate exposed 2.5 hrs. near apparently healthy bed located 300 ft. from bed in which fungus was sporulating freely	Sporangia found

TABLE 1.—(Continued)

Date	Locality	Material examined	Results
4-23-33	Wilson, N. C.	Tobacco leaves from apparently healthy bed	Sporangia found
"	"	Tobacco leaves from apparently noninfected bed 4 miles west of Wilson	" "
"	Bailey, N. C.	Tobacco leaves from apparently healthy bed	" "
"	"	Young pine branch collected between Bailey and Middlesex	" "
"	Middlesex, N. C.	Young pine branch. Few diseased plants in bed 90 ft. distant	" "
"	Wakefield, N. C.	Young pine branch. Diseased bed 1 mile distant	" "
"	"	Young pine branch. Mildew just appearing in an occasional bed in this district	No sporangia found
"	Durham, N. C.	Glycerine plate exposed 24 hrs. 7 miles east of Durham between Durham and Wake Forest	" " "

point distant from diseased beds. The situation chosen for this purpose was the Tobacco Experiment Station, Oxford, N. C. There were 2 foci of infection about 10 to 12 miles distant, one southeast and the other southwest of Oxford. Glycerine plates were exposed for 24 hour periods for 11 days prior to the outbreak of the disease. Seedlings were collected daily and their leaves examined for the presence of sporangia. The data for this series of examinations are assembled in table 2.

It will be seen that sporangia were first trapped at Oxford, N. C., on April 25. A period of weather that was continuously very favorable for the development of sporangia and their spread occurred from April 19 to April 22. The first evidence of infection in the seed beds at Oxford was noted on May 2, making an interval of 7 days between the date when sporangia were first found and that when a new crop of sporangia had formed. It should be recalled that this interval corresponds with the cycle of development of the disease.

#### SOURCES OF INOCULUM IN SPRING

In previous investigations, three sources of inoculum for the initiation of the disease in spring have been suggested: (1) old tobacco plants that survived the winter, (2) infected seed, and (3) infested soil.



In parts of Australia (2) many plants survive the winter and, in spring, produce suckers upon which downy mildew lesions appear. While all

TABLE 2.—*Dissemination of sporangia by air currents, and its relation to the development of downy mildew at Oxford, N. C.*

Date	Material examined	Place collected	Results
4-21-33	Tobacco leaves	Seed bed on new site	No sporangia found
"	" "	" " " old "	" " "
4-22-33	Glycerine plates (3)	North, south and east of seed beds	" " "
"	Tobacco leaves	Seed bed on new site	" " "
"	" "	" " " old "	" " "
4-24-33	Glycerine plates (3)	North and south of seed beds	" " "
4-23-33	Tobacco leaves	Seed bed on new site	" " "
"	" "	" " " old "	" " "
4-25-33	Glycerine plates (4)	North, south and east of seed beds	Sporangia found
"	Tobacco leaves	Seed bed on new site	" "
"	Glycerine plates (4)	North, south and east of seed beds	No sporangia found
"	Tobacco leaves	Seed bed on old site	Sporangia found
4-26-33	" "	" " " new "	" "
"	" "	" " " old "	" "
4-27-33	" "	" " " new "	" "
"	" "	" " " old "	" "
4-28-33	" "	" " " new "	" "
"	" "	" " " old "	" "
4-30-33	" "	" " " new "	" "
"	" "	" " " old "	" "
"	Glycerine plates (3)	North, south and east of seed beds	" "
"	" " (3)	North, south and east of seed beds	" "
"	Flowers of <i>Azalea nudiflora</i>	Within 5 ft. of seed bed	" "

aboveground parts of tobacco plants are killed by frosts, except in occasional years, throughout the flue-cured tobacco area, it is of common occurrence for suckers to arise in spring from the underground portions of stubbles that have remained alive. Because of the mild winter, plants remained

alive in eastern North Carolina until March, 1932. Such plants therefore may have served as sources of inoculum for the wide-spread, early infections in seed beds that season.

Angell (1) was led to conclude that infected seed is an important source for the initiation of the disease in seed beds in Australia because of the occurrence of lesions on capsules, the presence of coenocytic mycelium in the seed from such capsules, the early appearance of the disease on seedlings grown from infected seed, and the occurrence of mildew on tobacco 20 miles away from the nearest plantation. No evidence has yet been found that such is the case within the United States.

During a two-week period in late March and early April, 1933, isolated outbreaks of the disease occurred throughout North Carolina in the localities indicated in figure 4. Doubtless numerous other outbreaks occurred to which our attention was not directed. These early outbreaks, that occurred a month or more prior to the season for transplanting, may be noted to be distributed rather generally throughout the tobacco-growing section. Investigation of these outbreaks established the fact that the seed beds in which downy mildew first appeared were located on or near sites occupied the preceding season by infected beds. This suggests the possibility that downy mildew may have survived the winter in the debris remaining from diseased seedlings grown in 1932. This assumption is strengthened by the facts that the pathogen forms oospores within the tissues of decaying leaves and that oospores of downy mildews generally, are known to constitute the hibernating stage.

Since it has not been possible thus far to germinate the oospores of the tobacco downy mildew, no definite knowledge is available regarding their period of dormancy, the conditions for their germination, and their relation to the initiation of the disease on seedlings. It is believed, however, that the fungus hibernates as oospores in occasional old seed beds and that oospores are the most important source of inoculum for the initiation of the disease in spring. Evidence in support of this belief is based (1) on the observation that the earliest occurrence of the disease, in 1933, was in beds located on sites utilized for seed beds during 1932; and (2) on the results of surveys to determine the extent and rate of spread of the disease from these beds. Surveys were made of areas surrounding foci of primary infections in three cases. It should be recalled that sporangia have previously been shown to be air-borne. The results of each survey were similar to the one shown in figure 5, made in Granville County. In this case the disease remained confined to the old bed, in which it first appeared about April 7, until the last few days of that month. On April 24 there was no evidence of downy mildew in any other seed beds in the area shown in figure 5. The period from April 19 to 22 was, however, very favorable

for sporulation. When this area was next surveyed, on April 30, the disease had attacked all the plants in a bed about 50 yards distant from the old bed that served as the primary center, and it was just beginning to make its appearance in the others indicated on the map. Most of these seed

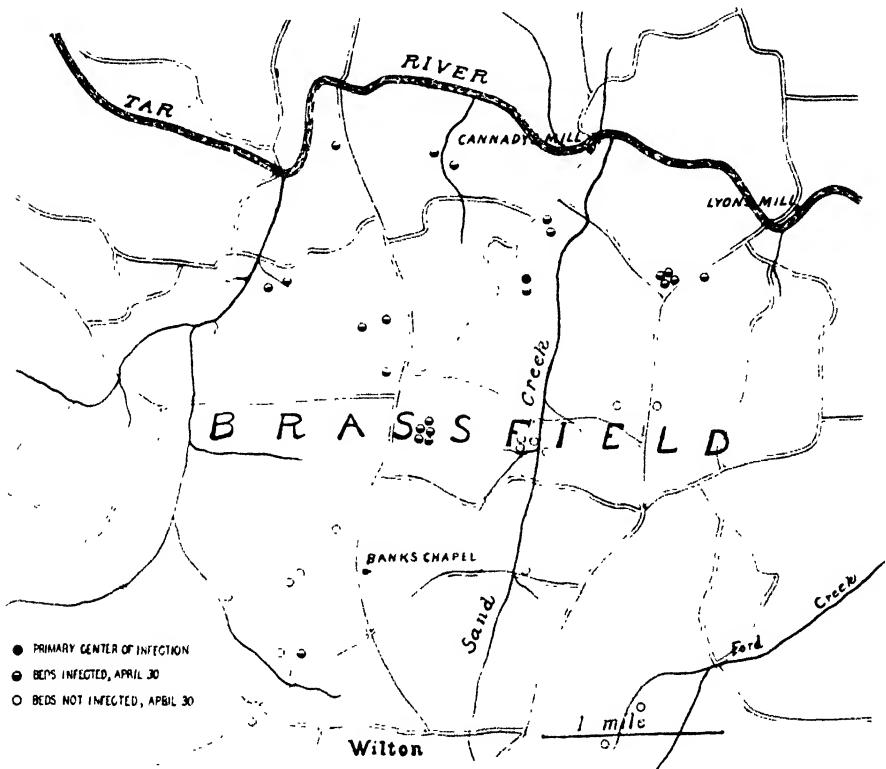


FIG. 5. A portion of Brassfield township, and adjacent territory in Granville County, N. C., showing the locus of a primary center of infection. The position of other seed beds in which infection was evident on April 30 is also indicated.

beds were in a northwesterly direction from the primary center. No evidence of infection was found in seed beds situated north of the Tar River. Two days later, however, the disease was beginning to appear, not only throughout this entire area, but was scatteringly present in seed beds 10 to 15 miles distant.

#### GERMINATION OF SPORANGIA

The sporangia of *Peronospora nicotianae* are extremely variable in their capacity for germination. Among the factors that influence germination are moisture, temperature, light, and reaction of the medium. Germination

cannot take place except in a film of water or in a saturated atmosphere. If sporangia are powdered onto a slide and placed in a container with a relative humidity of 95 per cent, they will not germinate. Ordinarily, when freshly formed sporangia are placed in drops of water on microscope slides, and are maintained at about 70° F. from 50 to 90 per cent will germinate within 5 hours. A single, lateral, crooked or tortuous germ tube usually is formed, (Fig. 3, B), whose growth soon ceases. At 45 to 60° F. germination is accomplished within 2 hours. Sporangia maintained for 22 hours at 79° F. had not germinated, but a portion of them were found to be still viable. When exposed for 8 hours at 82° F. the sporangia were still capable of germination, but were killed by exposure to this temperature for 42 hours. When freshly formed sporangia were powdered onto dry slides, however, and exposed, in darkness, to a temperature of 84° F. for 1 hour they were found incapable of germination when placed under conditions favorable for germination.

Sporangia germinate as readily in diffuse light as in darkness, as shown in a series of trials. When, however, sporangia are kept continuously in drops of water in a container placed on a block of ice and are exposed to direct sunlight, they are incapable of germination after an hour's exposure. Angell and Hill (2) found that sporangia lost their viability in less than an hour's exposure to direct sunlight, at a time when the shade temperature was 23° C.

Malic acid and bicarbonate of soda in aqueous solution were used in testing the effect of reaction of the medium on germination of sporangia. A series of solutions whose pH concentration, as determined electrometrically, ranged from pH 3.63 and pH 8.8 were employed. The sporangia germinated in each of the different concentrations, but germination was most vigorous and appeared to be stimulated throughout the acid range. In the alkaline range the germ tubes were short and the percentage of germination was much reduced.

#### VIABILITY OF SPORANGIA

Consideration was given to some of the factors that conceivably influence the length of time that sporangia of *Peronospora nicotianae* remain viable, because of the fact that they are subjected to varying environmental conditions between the time that they are mature and find lodgment after having been carried through the air. Seed beds in which they lodge are, in certain instances, known to be some miles distant. The factors concerned include temperature, light, and age of the sporangia.

In general, the sporangia are short-lived, approximately one per cent only being viable after 4 to 7 days. Angell and Hill (2) report as an extreme case the viability of sporangia 117 days old, after they had been kept

in air-dry soil at a temperature of 3° to 5° C. Differences in relative humidity appear to be of no significance in modifying viability. A series of relative humidities ranging from 10 per cent to 95 per cent, were maintained in desiccators over different percentages of sulphuric acid. As a result of several series of trials maintained at a temperature of 76° F., it is concluded that sporangia are equally viable after exposure to any humidity within this range. If sporangia are subjected to a temperature of 79° F. under conditions of saturation they begin to lose their viability after about 21 to 24 hours. With longer exposures, there is a rapid increase in the death rate. With lower temperatures there is an increase in the length of time that sporangia remain viable, indicating that longevity is correlated with low temperature.

Angell and Hill (4) found that sporangia stored under moist conditions, at 18° C., remain viable for 2 months, and when stored over fused calcium chloride at this temperature (3), for 5 weeks.

As previously shown, direct sunlight is lethal to sporangia exposed for an hour. When sporangia from the same source as those exposed to direct sunlight are stored at a constant temperature and are exposed to diffuse light they remain viable for several days.

#### RELATION OF METEOROLOGICAL CONDITIONS TO THE OCCURRENCE OF DOWNY MILDEW

Unsuccessful attempts were made to study the course of the disease under conditions of constant temperature, 76° F., and at each of the following relative humidities: 55, 65, 75, 85, 95, and 100 per cent. Potted tobacco plants were inoculated by wetting the leaves with a suspension of sporangia. They were then sealed under bell jars and a stream of air, conditioned by being bubbled through appropriate concentrations of sulphuric acid, was continuously passed through these jars. By adjustment of the rate of flow of the conditioned air, fairly constant relative humidities were maintained, as shown by psychrometers suspended inside the bell jars. If the films of moisture were permitted to remain until infection had been accomplished, lesions developed. No fructifications of the pathogen appeared, which was doubtless due to the high temperature.

Observations indicate that the course of the downy mildew is closely circumscribed by weather conditions. This fact might be anticipated from the results of studies previously recounted on the effects of environmental factors on germination and viability of sporangia. Additional evidence bearing on weather as a modifying factor was obtained by means of a Friez hygrothermograph. This instrument was placed within a tin box with numerous perforations in the sides and ends to permit easy air circulation. A cloth or a sheet of Celotex was placed on top of the tin box to prevent

heating by direct radiation. This box was placed upon the ground within the seed bed. In addition, records were made of the character of the sky and of rainfall.

During a 7-day period, beginning April 15, the instrument was placed in an apparently healthy bed, that was located about 25 yards away from a bed that was first noticeably infected about March 30. Several small spots developed during the week in which the instrument was in this bed. In the following week, beginning April 23, the hygrothermograph was

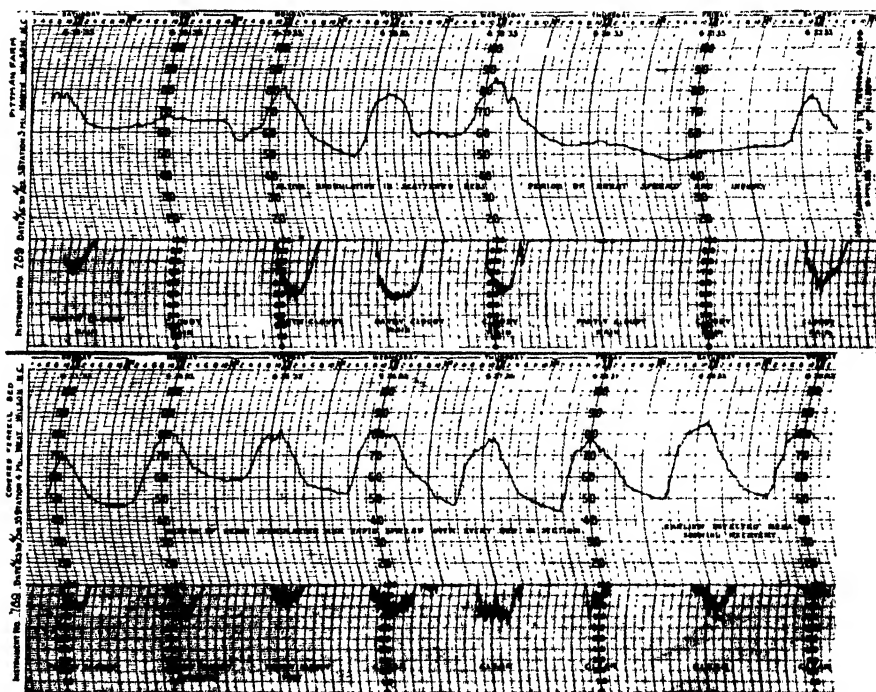


FIG. 6. Meteorological record for near Wilson, N. C., for the period from April 15 to April 30, 1933. The hygrothermograph was placed within the seed beds indicated.

placed in another bed about 5 miles distant. Examination of the leaves of the seedlings in this bed showed that sporangia were present, but there was no evidence of infection. Infection was first apparent the preceding day in a bed about 100 yards distant from that in which the instrument was placed. In this case downy mildew developed throughout the entire bed during the week in which the instrument was located in it.

The meteorological record for this 2-week period is shown in figure 6.

Attention should be called to the period from the evening of April 19 to the morning of April 22, when the temperature remained below 60° F.,



FIG. 7. Meteorological record for the Tobacco Experiment Station, Oxford, N. C., for the period from May 1 to May 17, 1933.

the sky was almost continuously overcast and the relative humidity remained at the point of saturation. These conditions are optimum for sporulation, dissemination, and infection; and, in consequence, the disease appeared in every bed in the section during the fore part of the following

week. It may also be noted that temperature and humidity conditions favored sporangial production every night during the week beginning April 23. The 5 successive, clear days of the last of the week with maximum temperatures of approximately 80° F. were favorable, however, for the rapid growth of infected plants.

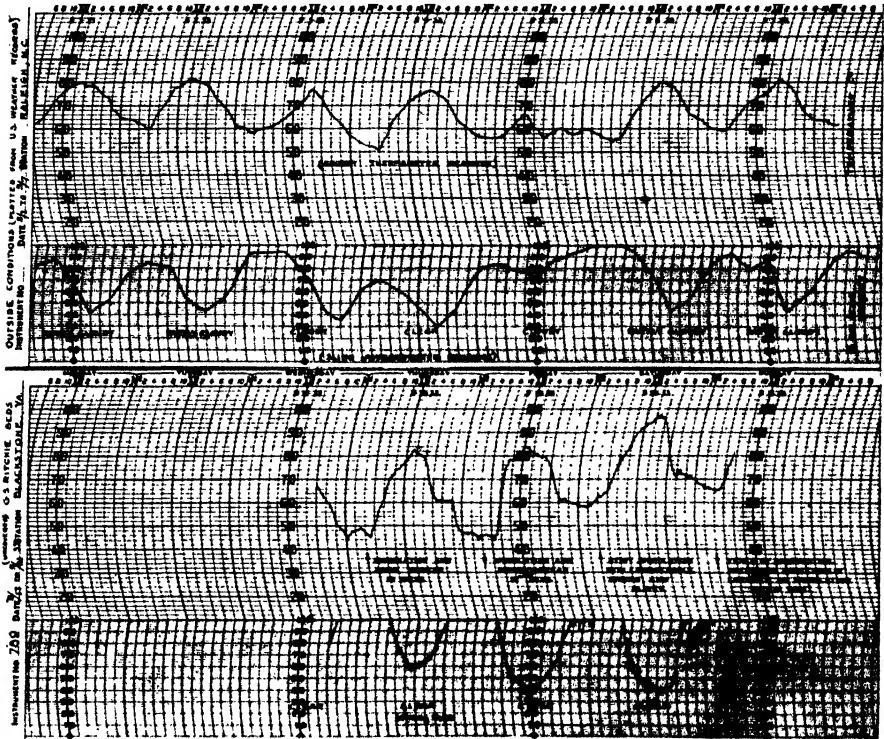


FIG. 8. Meteorological records. The upper graph is taken from data collected at the U. S. Weather Bureau Station, Raleigh, N. C. Compare it with the corresponding period in figure 7. The lower is a hygrothermographic record made within a seed bed at Blackstone, Va., May 17 to 21, 1933.

The hygrothermograph was placed in a seed bed at the Tobacco Experiment Station, Oxford, N. C., for the period from May 1 to 17, inclusive. The meteorological data given in figure 7 show the conditions that prevailed from the first appearance of downy mildew throughout the course of the disease and extending to the eventual recovery of the seedlings.

It may be noted that infection was first evident in some experimental seed beds in the garden in the morning of May 2. On the following morning it made its appearance in the seed beds, located about a half mile distant, from which the crop was planted. The disease did not assume



epiphytotic proportions until the period extending from the evening of May 4 to the morning of May 7, when the temperature almost continuously approximated 60° F. and the relative humidity remained at the point of saturation. This "downy mildew" weather was followed by a week favorable for the growth of tobacco, but with day temperatures between 80 and 90° F. that were unfavorable for the pathogen. The first three nights of this week were quite favorable for sporulation but only a slight amount of sporulation occurred subsequently. The period from April 11 to 17 was one in which temperature and rainfall favored the rapid growth and recovery of the seedlings. Hail, which fell during the afternoon of April 16, lowered the night temperature sufficiently so that sporulation again occurred in a near-by, late-sown bed that contained very young seedlings. The temperature of the 2 following days remained below 80° F., the temperature at night fell to between 50 and 60° F., and there was abundant production of sporangia on the young plants.

The temperature and humidity conditions recorded by the U. S. Weather Bureau Station at Raleigh, N. C.,<sup>4</sup> are not taken at the surface of the soil and should not be expected to correspond with those shown by the Friez hygrothermograph placed within seed beds. These differences are typified by comparison of the record for May 1 to 7, shown in figure 8 with that of the corresponding period at Oxford, N. C., about 40 miles distant from Raleigh, shown in figure 7.

The temperatures shown by the graph in figure 8 were plotted from hourly readings and the humidity graph from sling-psychrometer readings made at intervals of 4 hours; namely, at 4 and 8 in the forenoon, at noon, at 4 and 8 in the afternoon, and at midnight. The temperature graphs for these two places correspond more nearly than do the humidity graphs. The discrepancies in humidity can be explained if it is recalled that dew usually collects in tobacco seed beds at sunset and the seedlings remain covered with drops of dew all night long. Droplets of water remain on the lower leaf surface, moreover, for the greater portion of the day, unless the wind is blowing rather vigorously.

Downy mildew first appeared at Blackstone, Va., about May 14. On the nights of May 17, 18, and 19 (Fig. 8), meteorological conditions were ideal for sporulation. On the 20th the seed beds had the appearance of having been scalded. Temperatures in excess of 90° F., which prevailed that day between 11:00 A. M. and 5:00 P. M., together with unobstructed sunshine, effectively checked the further spread of the disease. A meager crop of sporangia were formed on the night of April 20. Subsequent observations showed that in the vicinity of Blackstone, Va., the disease disappeared al-

<sup>4</sup> The complete weather records for March, April, and May, 1933, were made available for study through the courtesy of Mr. L. A. Denson.

most as suddenly as it had come, and after 2 or 3 additional hot days those tobacco seedlings that survived were well on the way to recovery.

The lethal effects on sporangia of temperatures of 84° F. and above and of direct sunlight, as previously shown, account for the disappearance of mildew with the advent of summer weather. Although the organism had almost disappeared from the vicinity of Durham, N. C., by May 15, it was kept alive under "artificial" conditions, with the production of a crop of sporangia as late as July 11. This was accomplished by digging a pit at the edge of the woods, putting blocks of ice into the pit, and then placing potted inoculated plants directly upon the ice. By transferring fresh sporangia as they appeared, to healthy tobacco seedlings, and returning them to this "refrigerator," the pathogen has been made to pass through its pathogenic cycle repeatedly, even though the maximum daily temperature was 85° F., or above for 42 of the 56 days from May 15 to July 11, and 90° F. or above for 29 days of this period.

#### PREVENTION AND CONTROL

Satisfactory prevention and control of downy mildew of tobacco seedlings has not been accomplished. Nevertheless, it is indicated from the investigations that have been made and from observations that certain practices should have a palliative influence. The fact that the disease appeared in seed beds of all types of construction now employed suggests the need for experimentation on this phase of the problem. Tightly constructed seed beds with covers of closely woven texture stimulate rapid growth of seedlings, and interfere with proper ventilation. Plants grown in such beds are apparently most liable to severe injury. In order to facilitate ventilation, the employment of narrow beds and the early removal of the frames is indicated. The slight elevation of such beds above the level of the surrounding soil should also be beneficial.

Sites on high ground are to be preferred to low lying, swampy situations; sites in open fields, to those closely surrounded by woods; and beds on steep slopes, exposed to good opportunities for air drainage, to those on level situations. More severe injury occurs in seed beds that are shaded during a portion of the day than in those more distant from the woods.

Thinly sown beds and those containing less than half of the usual stand of seedlings suffer less damage than thickly sown beds.

It is customary to remove the seed-bed covers a few days or at most a week prior to the time for transplanting. When the covers are removed 3 or 4 weeks before the season for transplanting and are returned at night only if frost is indicated, the seedlings are more hardy and less subject to serious damage.

The influence of temperature on sporulation and on viability of spo-

rangia indicate experimentation on artificially heated beds. This is also indicated by the necessity of moisture for germination and infection. Angell and Hill (2) have suggested the use of seed beds so constructed as to make possible the control of temperature and humidity.

Seed beds should be made in new situations each year. The use of sites occupied by beds during the previous year apparently constitutes the most important source of primary infection. This observation is probably the most significant one in the present investigation. Beds on old sites may be expected to become infected about 3 or 4 weeks prior to the general prevalence of downy mildew. Such beds are continuous sources of sporangia that are spread by air currents to the nearest seed beds and thence to more distant ones until eventually all seed beds are infected. Universal avoidance of old bed sites may be expected to be an effective preventive procedure.

The destruction of all tobacco seedlings and debris that remain in the seed bed, as soon as the crop is transplanted, is indicated by the fact that *Peronospora nicotianae* forms oospores.

When the disease appears, applications of nitrate of soda should be made to stimulate the recovery of diseased seedlings.

Transplanting seedlings from infected beds is counter-indicated. Instead, they should remain undisturbed until after recovery is advanced to the stage that new leaves and new roots have formed.

#### SUMMARY

Downy mildew or blue mould of tobacco has for years been seriously destructive to the crop, especially to seedlings in Australia. In 1931 it appeared in the Florida-Georgia district in epiphytotic proportions. It recurred in the two subsequent years and spread so that it now is ubiquitously present throughout the southeastern United States in the area devoted to the culture of flue-cured tobacco. It has also extended its range into the Burley tobacco-growing sections of Tennessee and western North Carolina and into Maryland and Pennsylvania.

Many species of *Nicotiana* and many agronomic varieties of cultivated tobacco are subject to attack. It is pathogenic, in addition, to seedlings of tomato, pepper, and eggplant.

Downy mildew of tobacco is a necrotic disease. Small seedlings succumb to its attack; many older ones survive. Fructifications of the pathogen form a copious downy covering over the lower leaf surface, the most characteristic sign by which the disease may be identified.

The disease is caused by *Peronospora nicotianae* Speg. Its pathogenic cycle covers from 4 to 7 days. Sporangia are produced in the interval between daybreak and sunrise, and appear a few hours before death and col-

lapse of the invaded tissue. Oospores form within the dead tissues and are mature 4 to 7 days after death of the cells. Nothing is known of their germination.

The germ tubes enter by way of the stomata, and the mycelium is intercellular. Digitate haustoria penetrate the cells. Toxic water-soluble substances are formed within diseased tissues and their presence accounts, in part, for the high mortality rate when diseased seedlings are transplanted.

Sporangia have been trapped at long distances from diseased seed beds, which shows that they are air-borne. By means of spore-traps, sporangia were found at one location a week prior to an outbreak of downy mildew.

Seed beds situated on or near the sites occupied by old seed beds have been found to constitute primary centers of infection in early spring.

Moisture is necessary for germination of the sporangia of *Peronospora nicotianae*. Exposure for about an hour to direct sunlight is lethal. Germinations have not been accomplished at 79° F. or above. Continuous exposure of sporangia to a temperature of 82° F. and exposure for an hour at 84° F. destroys their viability. Sporangia are relatively short lived, except at low temperature, and viability does not appear to be modified by storage at different relative humidities.

Meteorological conditions exert a profound influence upon the course of the disease. The disease is checked by clear days with temperatures of 84° F. and above. Rainy weather, with continuously overcast skies and temperatures of 50 to 60° F., is ideal for sporulation and for the dissemination and germination of sporangia. *Peronospora nicotianae* has been made to repeat its pathogenic cycle under artificial environmental conditions.

Methods of prevention and control have not been determined. The avoidance of placing seed beds on sites utilized for seed beds during the previous year should be of primary importance, if universally practiced. It is recommended also that plants and debris remaining in seed beds after the crop has been transplanted be destroyed. The beds should be thinly sown, should be situated to provide ample air and soil drainage, and the covers should be removed early to render the seedlings more hardy.

When downy mildew appears applications of nitrate of soda should be made to stimulate the recovery of the seedlings.

Transplanting should be delayed until the plants have recovered, since a large proportion of the seedlings perish if transplanted from seed beds in which the disease is actively developing.

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# TEMPERATURE STUDIES ON STRIPE OF BARLEY<sup>1</sup>

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## INTRODUCTION

Although stripe of barley caused by *Helminthosporium gramineum* Rabh. has been recognized and studied for many years, it was not until relatively recently that the environmental conditions affecting the development of this disease have received major attention. The development of suitable inoculation technique has made possible a more critical study of the environmental factors influencing stripe. Because spore production in culture on ordinary media has not been obtained, any method of inoculation involving conidia has depended upon infected plants. Natural infection during the early stages of kernel development has not been consistently high enough to depend upon this method for infected seed. Therefore, most of the earlier workers were dealing with infection percentages too low to make their results especially significant. Although mycelial production in culture has been abundant, difficulties in using this source of inoculum in seedling infection were not overcome until relatively recently.

The influence of temperature upon stripe development of barley has been discussed by various workers, beginning with Ravn (10). Several of the investigators have attempted to give a rather definite optimum temperature for disease production, varying from 0–8° C. by Isenbeck (6), to 15° C. by Leukel, Dickson, and Johnson (8). The differences in reported optimum temperatures may be accounted for in part by different technique, host varieties, and cultures of the parasite used in the several investigations. However, as stated earlier, the relatively low stripe infection obtained by the earlier workers has made much of the early temperature studies difficult to interpret. The results here presented would indicate that it was impossible to specify any condition as optimum unless such modifying factors as method of inoculation, host variety, culture of the parasite, etc. were defined specifically in relation to the variable environmental factor under consideration.

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<sup>2</sup> The writer wishes to make grateful acknowledgement to Dr. J. G. Dickson for advice given during the course of study, and for aid in the preparation of the manuscript. He also wishes to thank Prof. B. D. Leith for counsel during the investigation, Miss Helen Johann for criticism of the manuscript, and Mr. Eugene Herrling for aid in the preparation of the illustrative material.

RELATION OF TEMPERATURE TO GROWTH OF *HELMINTHOSPORIUM*  
*GRAMINEUM* IN CULTURE

Five strains of *Helminthosporium gramineum* selected from widely different geographical regions were grown in 2 series on potato-dextrose agar at constant temperatures from 8° to 32° C. arranged at 4-degree intervals. The 5 cultures were selected on the basis of differences in pathogenicity and apparent differences in temperature relations with reference to disease production. The cultures used were: (1) 21a1, a monoconidial culture, isolated at Madison, Wisconsin; (2) 41, a culture of monoconidial origin from Lincoln, Nebraska; (3) Selo 2, a culture of monoconidial origin from Detskioe Selo, near Leningrad, Russia; (4) Ariz. 1, a mycelial isolation from Arizona; and (5) Ind. 1, also a culture of mycelial isolation from La Fayette, Indiana. Approximately equal bits of mycelium of the same age were transferred to each Petri dish; the inoculated dishes were then placed immediately in chambers operating at the temperatures desired. Two series of 5 plates each were employed in determining the average colony diameter. The diameter of each colony was measured at the end of 6½ days after inoculation. The average diameter is recorded in table 1. Mycelial growth

TABLE 1.—*Effect of temperature on colony diameter of cultures of Helminthosporium gramineum when grown on unacidified potato-dextrose agar in Petri dish cultures. Diameter of colonies were determined 6½ days after starting. Average of 2 series of 5 plates each*

Culture of fungus	Diameter in millimeters at temperatures given in degrees Centigrade						
	8	12	16	20	24	28	32
21a1	11.4	26.6	43.9	53.7	77.4	83.8	61.1
41	12.9	28.3	47.3	60.9	78.1	82.2	27.1
Selo 2	7.0	15.3	34.0	34.6	51.7	51.2	19.0
Ariz. 1	13.1	30.2	46.3	58.1	77.5	78.7	38.2
Ind. 1	14.8	28.6	43.0	58.3	76.5	72.4	43.5

as measured by colony diameter was slow at 8°, increased rapidly with rising temperatures until 24° was reached, slowed up in growth rate with minor cultural changes at 28°, and dropped off markedly at 32° C. There was little difference in the temperature-growth response from 8° to 24° inclusive in the colonies of the cultures other than the Selo 2 isolation. At 28° the diameters of cultures 21a1, 41, and Ariz. 1 increased above those at 24°, while Ind. 1 and Selo 2 decreased slightly. The minimum temperature was considerably below 8° C., the lowest temperature used in the series. The optimum temperature on potato-dextrose agar was near 25° C. as Ravn

(10) reported when he used average daily increase in culture diameter on beer-wort agar for determining cardinal temperatures of growth. The maximum temperature was not quite reached at 32°, the highest temperature used in the study. Culture 21a1 seemed more tolerant of the higher temperature than any of the other isolations studied.

Modifications in the appearance of colonies, especially in the type of mycelium and in pigment formation were evident in addition to differences in diameter of the colonies at the various temperatures. The height and density of aerial mycelium in general, closely paralleled radial proportions, except the aerial mycelium of the Selo 2 isolation was scant at the lower temperatures, and Ariz. 1 was perhaps a little more dense, while the mycelium of 21a1 was tall and fluffy. In general, pigmentation of all cultures was least at the lower temperatures, and greatest at 32°. The different colonies showed considerable variation in intensity of color, and in zonation at the intermediate temperatures. Cultures 41 and Ariz. 1 formed pigment zones at 32 and 28°, and Ind. 1 formed growth zones at 16–28° inclusive. The shape of the colonies of 21a1, 41, and Ariz. 1 was almost circular at all temperatures, while Selo 2 and Ind. 1 were more irregular in circumference. The Selo strain produced the greatest variation in shape and general appearance. After 20 days' growth at 8°, colony diameters of cultures 21a1, 41, Selo 2, Ariz. 1, and Ind. 1 were 56, 59, 62.4, 68.8, and 72.8 mm. respectively. Culture 21a1 was the smallest at 8°, and largest at the higher temperatures.

#### INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF BARLEY

*Effect of Constant Temperature.* Although the study of the influence of a constant soil and air temperature upon the development of the barley plant has not been the main purpose of this investigation, a brief summary of the data taken upon the temperature responses of barley will aid in interpretation of the results obtained with reference to stripe expression after artificial inoculation. General notes were kept during the growth of several series, and specific data were taken on the uninoculated controls of one series after 40 days' growth.

Earliest germination of barley seedlings was favored by the temperatures of 28 and 32° C., where emergence took place in 3 to 4 days. The time of emergence was increased at 24, 20, 16, 12 and 8° C., respectively, 10–11 days being required for complete emergence at 8°. The growth and development of barley for the most part corresponded to that of spring wheat as described by Dickson (2). The coleoptile was longest at 16°, and shorter at both higher and lower temperatures. Likewise, the length of the sub-crown internode was greatest at 16°, and shorter as the temperature increased or decreased. The diameter of the base of the culms was largest at



20°, slightly less at 16° than at 20°, and smallest at 32° C. The first leaf was shortest at 8°, and increased in length with rise in temperature to 24°, beyond which no data were taken. The first leaf was widest at 12°, and markedly decreased in width with temperature increase. However the later leaves were widest at 20°, with gradual decrease in width at higher and lower temperatures. The number of mature leaves and approximate height of tops, after 40 days' growth was correlated with rate of growth which reached a maximum at 24° C. The dry weight of tops was greatest at 24°, with a secondary maximum at 16° C. The general appearance of the barley plants after 40 days' growth at the several temperatures is shown in figure 1.

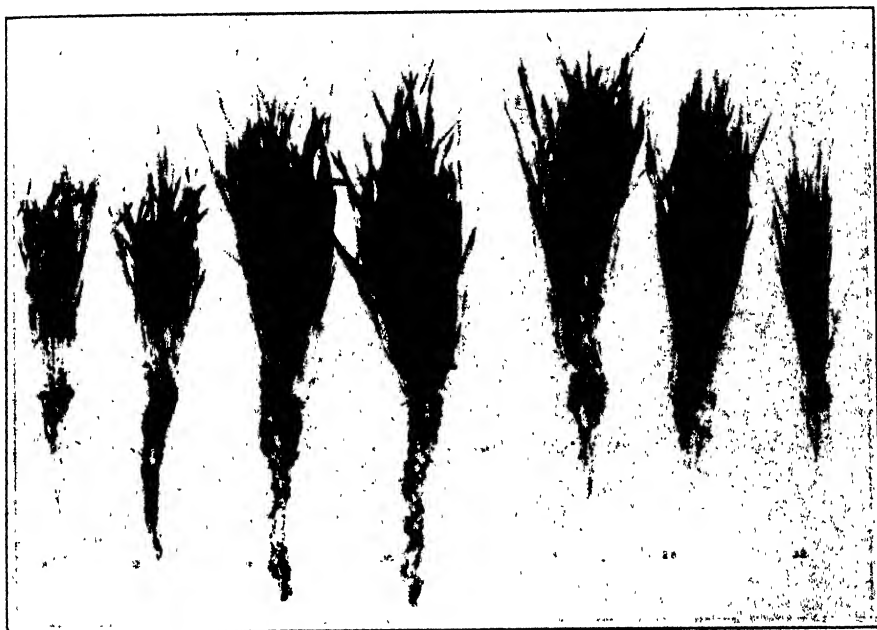


FIG. 1. Effect of soil and air temperatures on the development of Wis. Ped. 6-6 barley. Soil temperatures were maintained constant at temperatures indicated. Air temperatures were as follows: Plants at 8 and 12° soil temperature were grown in a greenhouse held at 8-12° C., 16° and 20° soil temperatures at 20° air temperature, 24° soil at 24° air, and 28 and 32° soil at 28° C. air temperature.

The length and dry weight of roots as compared with the tops of the various temperatures indicated a different temperature response for roots than for shoots of barley. The longest root growth was at 20°, with the heaviest dry weight at 12°, with only a slight decrease at 16° C. The largest total dry weight per plant, like dry weight of tops was produced at 24° where the tissues were relatively more mature than at the lower temperatures. Flow-

ering and maturation of grain in plants grown at the different temperatures progressed in the following order from first to last respectively: 24, 20, 16, 12, and 8° C. Abnormal flowering and very little grain production occurred at 28 and 32° C. As the seedlings increased in age, the temperature conducive to better top development gradually lowered. The greatest final development and yield of Wisconsin Pedigree 6-6 barley occurred at the lower temperatures 12-16° C., but the time required for completion of growth was likewise longer.

*Effect of Change in Temperature.* Change of temperature during the period of development of the barley plant had a marked effect on subsequent growth and appearance. When seed germinated and grew during the seedling stage at a low soil temperature and was later changed to a higher temperature the plants assumed a type of growth similar to that of plants that had grown continuously at the higher temperature. In like manner, when slender plants grown at a high temperature were transferred to a low temperature they soon developed a stocky growth not unlike those grown continuously at the lower temperature.

#### RELATION OF TEMPERATURE TO STRIPE DEVELOPMENT

*Method of Inoculation.* Very little had been accomplished toward developing a method of inoculation that afforded stripe resembling that caused by natural floral infection until Johnson (7) began investigation of the possibility of infection resulting from seed or seedling inoculations. DeHaan (5), Genau (4), Winklemann (11), Fuchs (4), and Majdrakoff (9) tried several methods involving seed, seedling, and floral infection but were unable to produce consistently high percentages of stripe. Isenbeck (6) was the first to develop satisfactory methods for stripe inoculation. Flowers inoculated with dry conidia gave higher infection than when using conidial suspensions in water for floral inoculations. Isenbeck (6) also deglumed barley seed and, while still moist, rolled them in spore dust. This was the most severe type of inoculation used, giving higher percentages of infection than floral inoculations. When he placed deglumed seed in contact with chopped mycelium on agar, the results corresponded to those obtained with floral inoculations, but did not agree with the conidial inoculation of the seed. J. J. Christensen suggested that infection might be secured by placing barley kernels with the hull intact in contact with the fungus growing on sterilized wheat kernels.

In the present work, floral inoculations were carried out in the summer of 1930 by placing glassine bags over groups of plants just before the heads emerged from the uppermost leaf sheath. The heads were sprayed with a suspension of conidia obtained from diseased plants. More than 80 per cent stripe infection developed from seed florally inoculated in this manner

in 1930; but less infection was obtained by the same method during the less favorable seasons of 1931 and 1932.

Seed inoculations were made by placing the kernels between layers of potato-dextrose agar on which the fungus was growing, followed by an overnight incubation before planting. Seed in hull, acid-hulled, and hand-hulled kernels were inoculated in this manner, with the former giving relatively little infection, and the latter two methods resulting in much higher infection. The acid-hulled seed resulted in the highest per cent infection. The method of acid hulling modified from that of Briggs (1) was as follows: Barley kernels were stirred in concentrated sulphuric acid until the lemma and palea were digested, except for a portion over the embryo and a very thin layer over the remainder of the seed. The kernels were then quickly poured into a paraffin-coated wire basket, rinsed in cold water, and immersed in dilute alkali. The time required for the acid digestion of the hulls without injuring germination varied with the variety or selection of barley, and probably with the locality in which the seed was produced. The time required for acid digestion with samples of Wisconsin Pedigree 6-6 was 4 minutes for most of the experiments.

High percentages of seed infection were also obtained when kernels previously treated with 70 per cent alcohol for 15 minutes and rinsed in distilled water were inoculated by contact with cultures of the fungus growing on a wheat-kernel medium prepared by autoclaving equal parts by weight of wheat kernels and distilled water. This is essentially a modification of the method first suggested by Christensen in which he treated the barley kernels with an organic mercury dust prior to inoculation.

*Relation of Constant Temperature.* The influence of soil temperature on the development of stripe has been the subject of several investigations since the time of Ravn (10), who noted that more stripe occurred in early plantings of naturally infected seed than in later plantings from the same seed lot. Johnson (7) concluded that low temperatures favored infection, and that the critical period for infection was during, and immediately after seed germination. The total number of striped plants obtained was, however, so small as to make the data on temperature response inconclusive. Isenbeck (6) found relatively low temperatures, 0 to 8° C., and 25% soil moisture most favorable for infection and development of stripe. Leukel, Dickson, and Johnson (8) stated that the temperatures of 15° C. or below during the period of emergence favored stripe infection, and that temperatures above 20° C. inhibited the disease.

The temperature studies reported in this paper were conducted in the Wisconsin temperature tanks placed in greenhouses in which the air temperatures were controlled at 12, 16, 24, and 28° C. Soil composed of about 70-75% loam and 25-30% sand, with the moisture held within a range

TABLE 2.—*Influence of constant soil and air temperatures upon the development of stripe. Summary of experiments of 1931–1932*

Temperature in °C.		Determinations	Ped. 6-6 acid-hulled seed inoculated with culture		C.I. 5024 hull-less seed inoculated with culture	Ped. 6-6 florally inoculated <sup>c</sup>	Ped. 6-6 acid-hulled seed uninoculated control <sup>b</sup>
Soil	Air		21a <sup>a</sup>	41 <sup>b</sup>	38 <sup>c</sup>		
8	12	Total No.	152	127	86	63	60
		Striped No.	71	78	36	8	0
		Striped per cent	46.7	61.4	41.9	12.7	0
12	12	Total No.	191	139	86	64	75
		Striped No.	84	69	67	9	1
		Striped per cent	44.0	49.6	77.9	14.1	1.3
16	20	Total No.	225	152	82	66	93
		Striped No.	104	76	35	18	0
		Striped per cent	46.2	50.0	42.7	27.3	0
20	20	Total No.	229	155	86	61	71
		Striped No.	120	85	32	19	0
		Striped per cent	52.4	54.8	37.2	31.1	0
24	24	Total No.	191	144	78	62	67
		Striped No.	104	80	23	15	1
		Striped per cent	54.5	55.6	29.5	24.2	1.5
28	28	Total No.	219	142	83	61	88
		Striped No.	81	49	8	1	0
		Striped per cent	37.0	34.5	9.6	1.6	0

<sup>a</sup> Three series; <sup>b</sup> two series; <sup>c</sup> one series.

considered most favorable for plant growth, was used throughout the investigations. Three plantings of inoculated seed were made in the winter of 1931–1932 (Table 2). Wisconsin Pedigree 6–6 acid-hulled seed was inoculated with cultures 21a1 and 41, and C.I. 5024, a blue hull-less barley, was inoculated with culture 38, a monoconidial isolation from Wisconsin, by placing seed between layers of potato-dextrose agar on which the fungus was growing. The last named variety, C.I. 5024, and florally inoculated Pedigree 6-6 were included only in the third planting. The stripe resulting from the triplicate plantings of Pedigree 6-6 inoculated with isolation 21a1, and of the duplicate series of inoculations with culture 41 agreed as well as could be expected, and therefore are summarized in table 2. Pedigree 6-6 barley inoculated with cultures 21a1 and 41 resulted in 46.7 and 61.4% stripe respectively at 8° C., with a decrease at both 12 and 16°, and with an

increase at 20 and 24° C. The development of stripe in response to temperature was decidedly different when C.I. 5024 inoculated with culture 38, and 1931 Pedigree 6-6 florally inoculated seed were tested. The percentage of stripe obtained with C.I. 5024 was 77.9 at 12°, with much less both at 8° and at the higher temperatures. This barley variety showed a slight tendency to outgrow the stripe infection even though a lower leaf was distinctly infected. Florally infected Pedigree 6-6 showed a still different temperature reaction; the highest percentages of stripe developed at 20 and 16° C.

TABLE 3.—*Influence of constant soil and air temperatures upon the development of stripe. Summary of two series duplicated 1932-1933*

Tem- perature in °C.		Determina- tions	No. of plants used and stripe infection					
			Ped. 6-6 inocu- lated with culture 21a1		C.I. 5024 hull-less inocu- lated with cult- ure 38	White hull-less inocu- lated with Ariz. 1 cult- ure	×76-2- 6-1 florally inocu- lated	Ped. 6-6 florally inocu- lated
			Acid- hulled seed	Seed in hull				
Soil	Air							
8	12	Total No.	278	246	257	230	71 *	110 *
		Striped No.	156	199	174	153	11	14
		Striped per cent	56.1	80.9	67.7	66.5	15.5	12.7
12	12	Total No.	291	213	247	196	77 *	163
		Striped No.	142	177	113	101	23	21
		Striped per cent	48.8	83.1	45.7	51.5	29.9	12.9
16	16	Total No.	320	343	235	248	167	265
		Striped No.	140	229	47	63	60	56
		Striped per cent	43.8	66.8	20.0	25.4	35.9	21.1
20	16	Total No.	287	359	189	209	181	207
		Striped No.	114	201	17	23	41	31
		Striped per cent	39.7	56.0	9.0	11.0	22.7	15.0
24	24	Total No.	200	287	162	166	116	167
		Striped No.	94	155	7	14	26	25
		Striped per cent	47.0	54.0	4.3	8.4	22.4	15.0
28	28	Total No.	120	264	127	141	95	159
		Striped No.	30	78	0	3	9	4
		Striped per cent	25.0	29.5	0	2.1	9.5	2.5

\* Only one series represented.

The constant-temperature studies were continued in the winter of 1932-1933, and larger populations of plants were used. In addition to the 2 types of inoculations previously employed, kernels were inoculated by growing the fungus on autoclaved wheat kernels for 4 days, followed by placing barley seed in contact with the inoculum and allowing this mixture to incubate for 4 days. Acid-hulled Pedigree 6-6 seed, C.I. 5024, and White Hullless barley were inoculated by placing seed between layers of the agar cultures 21a1, 38, and Ariz. 1, respectively. Pedigree 6-6 Oderbrucker and X76-2-6-1, an Arlington Awnless type, florally inoculated were used (Table 3). There was 83.1 per cent stripe at 12° with a little less at 8°, and still less as the temperature increased, when culture 21a1, grown on wheat, was used as inoculum with Pedigree 6-6 nonhulled as the host. When acid hulled Pedigree 6-6 was inoculated with isolation 21a1, the results coincided with those of the previous winter, namely, a relatively high percentage of stripe at 8°, with less at 12, 16, and 20°, and more again at 24° C. The stripe reaction of C.I. 5024 was somewhat changed; the most disease developed at 8°, 67.7 per cent, and less as the temperature rose. The White Hull-less variety, infected with Ariz. 1 culture of the fungus, gave final infection percentages essentially similar to those of C.I. 5024. The 2 florally inoculated varieties gave still different results in that most striping occurred at the intermediate temperatures, with a decrease in stripe at both the lower and higher temperatures.

The weighted averages of the stripe percentages obtained during the 2 seasons' study in the controlled-temperature tanks are given in table 4. Acid-hulled Pedigree 6-6 seed inoculated with culture 21a1 developed 54.2 per cent stripe at 8°, and 50.7 per cent at 24°, with less at the intermediate temperatures and at 28°. The two hull-less varieties gave most stripe at the lowest temperature used; namely, 8°. When culture 21a1 of the fungus growing on wheat kernels was used as inoculum with Pedigree 6-6 barley kernels with the hulls intact, the highest percentage of disease occurred at 12° with diminishing amounts at the higher temperatures. The two florally inoculated varieties, summarized together, showed most striping near 16°. The results, however, would have been more convincing had greater infection occurred. Thus it follows that the method of inoculation, host variety, and the culture of the parasite have to be considered in stating the temperature which is likely to produce the most stripe. A composite average of the percentages obtained in the two years' study rather clearly showed that most striping was likely to develop at the lower temperatures when barley seed was artificially inoculated with mycelium of *H. gramineum*.

Time is a factor to be taken into account in analyzing the effect of the several temperatures upon the development of stripe. Frequent periodic harvestings of striped plants were made in the first series of 1932-1933 in

TABLE 4.—*Influence of soil and air temperatures on stripe. Summary of data obtained during 1931, 1932, and 1933*

Method of inoculation	Host variety	Culture of parasite	Percentage of striped plants grown at					
			°C. 8	°C. 12	°C. 16	°C. 20	°C. 24	°C. 28
Floral . . . . .	× 76-2-6-1 and Ped. 6-6	Conidial suspension	13.5	17.4	26.9	20.3	18.6	4.4
Seed incubated with mycelium on wheat . . . .	Ped. 6-6	21a1	80.9	83.1	66.7	56.0	54.0	29.2
Acid-hulled seed in contact with mycelium on agar . . . . .	Ped. 6-6	21a1	52.8	46.9	44.8	45.3	50.6	32.7
Seed in contact with mycelium on agar . . . . .	C.I. 5024	38	61.2	54.0	25.8	17.8	12.5	3.8
Seed in contact with mycelium on agar . . . . .	White Hull-less	Ariz.1	66.5	51.5	25.4	11.0	8.4	2.1
Composite average			54.8	50.6	37.9	30.1	28.8	14.4

an attempt to learn more accurately the time after inoculation when certain percentages of stripe appeared. The determination of the time in days required for the appearance of disease symptoms in the first half of the total of striped plants developing at the various soil and air temperatures in the first series was arbitrarily chosen as representing fairly accurately the rate of striping (Table 5). For each method of inoculation the slowest disease production resulted at 8°, with each succeeding higher temperature, including 20°, inducing more rapid striping than the next lower temperature. At 24° the rate of striping was about like that at 20°, except for minor changes. Although the data are not conclusive, the rate of striping seemed to be retarded at 28°. If the percentage of stripe is divided by the number of days required for the first half of the total striped plants to develop symptoms, securing in this way what may be termed a per cent-time index, the data show that 8° gave the highest index for C.I. 5024, 16° for White Hull-less, 24° for acid-hulled Pedigree 6-6, and 20-24° for the floral inoculations. High indices were obtained from 12 to 24° C. for Pedigree 6-6 when inoculated with fungus growing on wheat. The results may be stated in another way; by balancing the percentage of stripe against the rate of development, a "corrected optimum" may be determined, which

lies near 12–16° for the composite grouping of the methods of inoculation, host varieties, and cultures of the parasite.

TABLE 5.—*Time in days required for appearance of disease symptoms in half of the total striped plants developing at the various soil temperatures. Series I, 1932–1933*

Method of inoculation	Host variety	Culture of parasite	Number of days required for 50 per cent of total stripe to develop at temperatures of					
			°C. 8	°C. 12	°C. 16	°C. 20	°C. 24	°C. 28
Floral . . . . .	× 76–2–6–1 and Ped. 6–6	Conidial suspension	65	51	41	35	38	50
Seed incubated with mycelium on wheat . . . .	Ped. 6–6	21a1	47	32	28	24	25	27
Acid-hulled seed in contact with mycelium on agar . . . . .	Ped. 6–6	21a1	51	36	28	28	26	27
Seed in contact with mycelium on agar . . . . .	C.I. 5024	38	38	28	21	19	—	—
Seed in contact with mycelium on agar . . . . .	White Hull-less	Ariz.1	56	37	21	21	19	—

*Relation of Change in Temperature.* Because the plants at 8° C. were quite slow in striping in the first constant temperature series of the winter of 1931–1932, one can of plants was placed in a 24° house, where disease soon became evident, with a higher percentage of striped plants than in those held at 8°. This suggested the importance of a study involving periodic change in temperature at various stages of plant and disease development. Leukel *et al.* (8) state . . . “that the influence of soil temperature on the development of stripe does not extend beyond the time of emergence.” Three experiments were conducted; 2 in the season of 1931–1932, and a third in the winter of 1932–1933 with the object of determining the effect of temperature change on stripe development when acid-hulled Pedigree 6-6 seed was inoculated with culture 21a1. The kernels were inoculated by placing them between layers of potato-dextrose agar on which the fungus was growing, and were planted in 8-inch pots. Immediately after they were placed in greenhouses with air temperatures of 8–12° and 24°. In the first two series, pots also were placed in a 16° house. Plants started at 8–12° were changed to 24° at periodic intervals, and *vice versa*. Those started at 16°



were changed to both 8-12°, and 24°. As a check for each of the series, some of the plants were maintained at constant temperatures. The detailed data of the second series, which was representative of the 3 experiments, are recorded in table 6.

TABLE 6.—*Effect of temperature change upon the development of stripe with acid-hulled Ped. 1-6 seed, inoculated with mycelium of culture 21a1*

Temp. at which started, or at which grown. °C.	Temp. to which changed. °C.	Days after inoc. when change was made	Total number of plants striped at following days after inoculation														
			12	15	18	21	24	27	30	33	36	39	43	46	53	59	
10-12	24	5	2	7	10	21	30	36	43	47	51	53	54	56	56	56	
10-12	24	12			4	15	19	28	32	35	35	38	38	38	38	40	
10-12	24	21						9	18	25	28	32	33	33	35	35	
10-12	24	30						1	5	14	33	43	51	52	54	57	
10-12	Not changed							1	6	11	22	27	31	36	37	42	44
16	10-12	8				1	2	2	5	10	15	17	20	20	22	26	
16	24	8		1	7	12	16	20	21	23	23	23	23	24	24	25	
16	10-12	18					1	6	11	16	20	22	22	22	22	25	
16	24	18			2	5	10	32	37	39	42	43	44	46	46	47	
16	Not changed					1	6	21	24	27	27	27	27	28	28	30	
24	10-12	3						1	3	16	24	30	33	38	41	47	47
24	10-12	7							2	3	12	15	18	20	21	25	26
24	10-12	13			2	2	2	4	6	12	15	22	25	27	27	28	29
24	10-12	18		1	4	11	15	19	22	32	38	42	44	46	46	47	48
24	Not changed			1	4	8	14	22	24	28	31	32	34	35	35	37	37

As suggested in earlier experiments, stripe developed slowly at 8°, and very much faster at 24°, when the temperatures remained constant. When the inoculated plants were changed from lower to higher temperatures, the rate of disease appearance was stimulated, and when shifted from higher to lower temperatures, striping was retarded. When inoculated plants started at an intermediate temperature were changed to the lower one, development of symptoms was slower, and when changed to 24°, striping was much faster.

The time of disease appearance and the percentage of total stripe of the 3 series are summarized in table 7, and illustrated graphically in figure 2a. Group No. 1 was earliest in striping, developing 25, 50, and 75 per cent stripe in 18, 24, and 30 days, respectively. This group represents those plants started at a low temperature and shifted to the higher one 5-9 days after inoculation. Group No. 2, shifted from low to high temperature 12-14

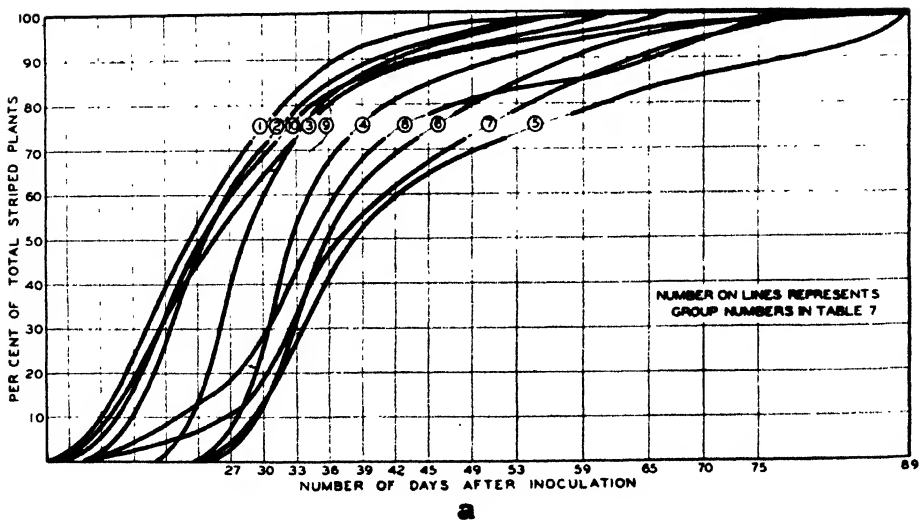
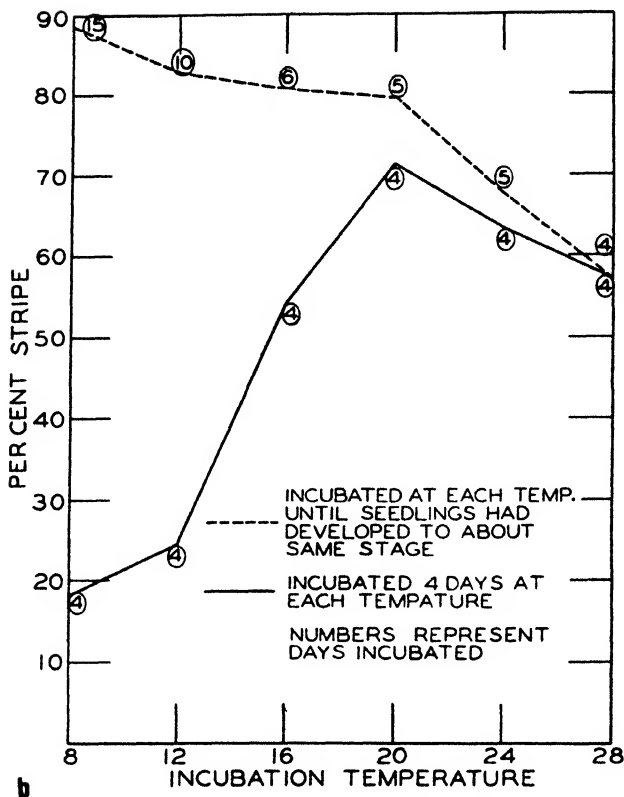


FIG. 2. The effect of temperature change and incubation period on stripe. (a) Influence of temperature change upon stripe development using acid-hulled Ped. 6-6 seed, inoculated with mycelium of culture 21a1. Summary of three series. (b) Effect of incubation temperature on stripe in Ped. 6-6 barley when inoculated with culture 21a1 grown on wheat.

TABLE 7.—*Effect of temperature change on stripe development using acid-hulled Pcd. 6-6 seed, inoculated with culture 21a1. Summary of 3 series*

Group No.	Temp. at which started or at which grown °C.	Temp. to which changed °C.	Days after inoc. when change was made	Percentage of striped plants at following days after inoculation																		
				12	15	18	21	24	27	30	33	36	39	42	45	49	53	59	65	70	75	89
1	8-12	24	5-9	2	15	23	34	53	66	76	83	87	91	93	98	98	98	98	100	100	100	100
2	8-12	24	12-14		10	29	48	63	73	78	81	87	87	87	94	98	98	99	99	100	100	100
3	8-12	24	16-22			1	16	39	56	76	81	87	89	92	97	100	100	100	100	100	100	100
4	8-12	24	30-42				1	6	27	51	64	74	83	85	88	89	98	98	98	98	100	100
5	8-12	Not changed					1	6	19	30	39	49	53	62	70	78	81	83	85	100	100	100
6	24	8-12	2-5				1	4	23	35	45	53	59	70	81	81	92	96	96	97	100	100
7	24	8-12	7-9		1	2	3	6	10	17	30	38	50	52	68	75	86	91	95	98	100	100
8	24	8-12	12-16		2	3	8	11	17	25	43	55	68	74	75	80	80	86	91	95	99	100
9	24	8-12	18-22		2	8	23	32	41	45	62	71	82	85	88	89	89	91	94	97	100	100
10	24	Not changed			1	5	16	34	51	59	70	81	82	85	86	91	92	94	98	100	100	100

days after inoculation, practically paralleled the stripe development in group No. 10, held constant at 24°; both were slightly later than No. 1. Group No. 9, changed from 24° to the lower temperature 18–22 days after inoculation was still a little later in striping than any of the groups previously discussed. Groups 3 and 4, shifted from low to high 16–22, and 30–42 days after inoculation were still slower in the initiation of striping; but thereafter, were much faster than any of the other groups, whether changed or constant. Groups 8 and 7, moved from high to low temperature at 12–16, and 7–9 days after inoculation, though striping began relatively soon, were quite slow in the relative rate of disease appearance, requiring 30 and 32, 38 and 39, and 49 and 50 days, respectively, for 25, 50, and 75 per cent stripe to occur. Group 6, shifted from a high to a low temperature 2–5 days after inoculation, reacted almost like that of group 5, which was held constantly at 8–12°, and was slowest in rate of striping. These results have been substantiated by using Pedigree 6–6 seed inoculated by placing it in contact with mycelium of culture 21a1 growing on sterilized wheat, followed by changes at periodic intervals from low to high, and from high to low temperatures.

The data accumulated with reference to the influence of temperature change upon the final percentage of striped plants appearing, presented in table 8, rather definitely indicated that less stripe developed when inoculated plants were changed from high to low temperatures than when shifted from low to high temperatures. In each of the first 4 groups changed from low to high, where the three experiments are considered collectively, the final percentage of stripe was greater than in any of groups 6–9 inclusive, which were started at 24° and later changed to 8–12°. This is likewise true of the 3 series taken separately, with slight exceptions in the third series. In comparing the amount of striping in each series, the highest disease percentage was in the first, with less in the 2 later series.

#### INFLUENCE OF INCUBATION TEMPERATURE ON STRIPE DEVELOPMENT

The investigation of the influence of incubation temperature on stripe development was approached from 2 angles: (1) seedlings were incubated after inoculation 4 days at the respective temperatures, after which the plants were grown in a greenhouse held at 16° C.; and (2) seedlings were inoculated and incubated until they reached approximately the same stage of development at each temperature, after which the plants were likewise grown in a greenhouse held at 16°. Pedigree 6–6 barley, inoculated with culture 21a1 growing on sterilized wheat kernels was used in the 2 duplicated experiments (Fig. 2b). The results of the 2 series incubated during a 4-day period at the several temperatures are given in table 9. The most stripe, 71.5 per cent, developed at 20°, less as the temperature increased or

TABLE 8.—The influence of temperature change on the final percentages of stripe development

Group No.	Temp. at which started or at which grown °C.	Temp. to which changed °C.	Days after inoc. when change was made	Series I			Series II			Series III			Total plants used		Average
				Plants used			Plants used			Plants used					
				Total No.	Striped No.	Striped per cent	Total No.	Striped No.	Striped per cent	Total No.	Striped No.	Striped per cent	Total No.	Striped No.	
1	8-12	24	5-9	42	38	90.5	102	56	54.9				144	94	65.3
2	8-12	24	12-14	29	22	75.9	91	40	41.0	90	41	45.6	210	103	49.0
3	8-12	24	16-22	62	44	71.0	77	35	45.5				139	79	56.8
4	8-12	24	30-42	63	43	68.2	105	57	54.3	90	40	44.4	258	140	54.3
5	8-12	Constant		88	53	60.3	97	44	45.4	84	38	45.2	269	135	50.2
6	24	8-12	2-5	58	27	46.6	108	47	43.5				166	74	44.6
7	24	8-12	7-9	57	31	54.4	86	26	30.2	88	39	44.3	231	96	41.6
8	24	8-12	12-16	58	34	58.6	91	29	31.9	81	38	46.9	230	101	43.9
9	24	8-12	18-22	29	18	62.1	114	48	42.1				143	66	46.2
10	24	Constant		46	33	71.7	95	37	38.9	66	48	72.7	207	118	57.0

decreased, with the least stripe, 18.7 per cent, at 8°. However, when the seedlings were incubated until they reached approximately the same stage of development, the stripe reaction was markedly different (Table 10). The number of days incubated at 28, 24, 20, 16, 12, and 8° was 4, 5, 5, 6, 10, and 15, respectively. The highest disease percentage developed at 8°, with a decrease at each succeeding increase in temperature. By compensating for increased rate of development at the higher temperatures with a longer in-

TABLE 9.—*Influence of incubation temperature on stripe development. Seedlings incubated 4 days at 6 different temperatures; the plants were then grown in a greenhouse at 16° C.*

Series	Determinations	Temperature in °C. at which incubated					
		8°	12°	16°	20°	24°	28°
I	Total No.	87	91	181	96	80	147
	Striped No.	9	21	93	64	56	89
	Striped per cent	10.3	23.1	51.4	66.7	70.0	60.5
II	Total No.	95	99	84	90	86	176
	Striped No.	24	26	51	69	50	95
	Striped per cent	25.3	26.3	60.7	76.7	58.1	54.0
Average	Total No.	182	190	265	186	166	323
	Striped No.	33	47	144	133	106	184
	Striped per cent	18.1	24.7	54.3	71.5	63.9	57.0

TABLE 10.—*Influence of incubation temperature and time on stripe development. Seedlings incubated to approximately same stage of development at each temperature and then maintained in a greenhouse at 16° C.*

Series	Determinations	Temperature in °C. and number of days incubated before planting					
		8° 15	12° 10	16° 6	20° 5	24° 5	28° 4 days
I	Total No.	79	66	74	86	65	147
	Striped No.	72	56	62	72	53	89
	Striped per cent	91.1	84.8	83.8	83.7	81.5	60.5
II	Total No.	91	191	99	153	87	176
	Striped No.	79	157	78	119	50	95
	Striped per cent	86.8	82.2	78.8	77.8	57.5	54.0
Average	Total No.	170	257	173	239	152	323
	Striped No.	151	213	140	191	103	184
	Striped per cent	88.8	82.9	80.9	79.9	67.8	57.0

cubation period at the lower temperatures, the resulting stripe was decidedly different from that produced when equal times of incubation periods were used.

#### DISCUSSION

In analyzing the effect of temperature on the development of stripe, such reactions as the effect of temperature on the host, on the parasite, and on the combination of the two in the production of disease must be studied. The temperature influence upon either plant alone presents a difficult problem for analysis that is made more complex when considered in relation to the phenomena of disease production. The final response of the host and parasite, or disease development, is the result of a series of interactions that are influenced by the successive stages of infection and parasitic invasion and the various responses on the part of the host. In studying temperature effects in relation to disease, the particular strain of the parasite, the peculiarities of the host variety used, the length of incubation period and temperature of incubation are some of the factors that the present study indicates should be taken into account. When constant temperatures alone were considered, the highest final percentage of stripe occurred at the lower temperatures; but when time was taken into account, the highest percentage of stripe per unit of time was found at the intermediate temperatures. When inoculated kernels were incubated until reaching approximately the same stage of development, the stripe reaction was similar to that of the plants held at constant temperatures. However, when the time of incubation was uniform, and temperature alone varied, the stripe reaction was decidedly different from the cases just mentioned in that the greatest percentage of stripe shifted to 20°. The change of temperature markedly affected both time of disease appearance and the rapidity of striping or the development of later symptoms. Whether under greenhouse or field conditions, the net result of infection in relation to temperature of incubation and changes in temperature is not alone measured by the percentage of stripe developing, but also involves the rate of development and severity of disease manifestation. From the practical standpoint, the final result is essentially the same in that all striped plants are worthless for grain production, but from the point of view of studying the influence of temperature upon the successive stages of striping, it is important to attempt a separation of these different factors and integrate them in terms of the detailed steps in disease development.

#### SUMMARY

The optimum temperature for the mycelial growth of *Helminthosporium gramineum* on potato-dextrose agar was found to be near 25° with a maximum above 32°, and a minimum considerably below 8° C.

Barley is a relatively low temperature plant, and with constant soil temperatures developed best near 12–16°. At these temperatures, barley developed more slowly than at the higher temperatures such as 20–24°, but the latter temperatures did not promote a balanced growth of the plants.

Floral inoculations were made by spraying a conidial suspension on flowers that had been enclosed within glassine bags about the time the heads emerged from the uppermost leaf sheath. Seed inoculations were made by, (1) placing seed between layers of potato-dextrose agar on which the fungus was growing, and (2), by incubating seed that had been placed in contact with mycelium of the fungus growing on steam-sterilized wheat kernels.

Several types of stripe reaction were evident when the different methods of inoculation, different cultures of the fungus, and different host varieties were used at the constant temperatures. Generally speaking, the lower temperatures induced the greatest percentage of striping when time was left out of consideration. The lower temperatures required longer for striping, which did not permit the definition of a clear-cut optimum. The relationship between time and the percentage of stripe development should be taken into account before definitely assigning an optimum temperature.

Change in temperature from low to high and high to low in the early stages of growth of barley and infection by the parasite markedly affected host and stripe development when seed was inoculated with mycelium. The change from low to high stimulated disease appearance, while the opposite change retarded disease expression. The final percentage of stripe was not greatly altered, but there was a tendency toward higher percentages of stripe when the plants were changed from low to high temperatures than when the opposite change was made.

In studying the influence of incubation temperature upon stripe development, the highest percentage of disease occurred at 20° C. when inoculated seedlings were incubated 4 days at all temperatures and later grown in a greenhouse at 16° C. The greatest percentage of stripe developed at the lower temperatures when inoculated seedlings were incubated until reaching approximately the same stage of development at each temperature and later grown in a greenhouse held at 16° C.

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# SOME FACTORS INFLUENCING GERMINATION OF SPORES OF PHYLLOSTICTA SOLITARIA

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## REVIEW OF LITERATURE

Although the apple-blotch fungus, *Phyllosticta solitaria* E. & E. has been the subject of much investigation, no thorough study of the effect of temperature and other factors on the germination of spores of this organism has yet been made.

Gardner (3) reported that spores of *Phyllosticta solitaria* germinated in water most rapidly at 75° to 80° F. (24° to 27° C.), but readily also at 68° and 61° F. (20° and 16° C.). Guba (4), in connection with considerable experimental work on the apple-blotch fungus, made some studies of spore germination. He used spores from pycnidia on leaves, fruit, and bark, putting them to germinate in sterilized water, tap water, rain water, and water containing crushed pieces of apple peelings, leaves, and bark. He found the percentages of germination to be small, irregular, and generally insignificant. He stated that sterilized distilled water was not a favorable medium, since germination was scant and germ tubes grew slowly, ultimately collapsing from want of nutrient, while in tap water, germination might reach 100 per cent. When spores from culture were placed in apple-fruit juice, weak sugar solution, sterilized water, or apple-bark extract, Guba found best germination in the solution first named and the degree of germination ranged in the order given. Spores, suspended in weak apple extract, from a 32-day old culture, germinated best at 25° C., while in those from a 46-day culture germ-tube development was most extensive and rapid at 25° and 30° C. Guba's results indicate the optimum temperature for spore germination to lie around 30° C. and rate of germ-tube growth to be greatest at 25° and 30° C. At lower temperatures, 15° and 20° C., the amount of germination was at first small and growth of germ tubes slow. Germination rarely occurred at 5° and 10° C. Kohl (5) carried out investigations of spore germination of *P. solitaria*. Sowings were made in various dilutions of citric acid, HCl, HNO<sub>3</sub>, sodium oxalate, H<sub>2</sub>O<sub>2</sub>, and boiled and fresh decoctions of apple fruits, apple leaves, apple bark, prune decoctions, and sugar solutions. Controls were carried out in sterilized distilled water. There was no evidence that any of the foregoing solutions stimulated germination.

<sup>1</sup> The writer wishes to express her sincere thanks to Dr. A. J. Mix for encouragement and advice during the course of this investigation.

## METHODS

Most of the spores used in this study were obtained from culture; some, however, were taken from twig cankers for purposes of comparison. Viable spores were obtained in culture by growing *Phyllosticta solitaria* on S/10 potato-dextrose agar<sup>2</sup>; and K.U. agar,<sup>3</sup> as recommended by Mix (8), and on K.U. agar with albumen substituted for potassium nitrate.

Cultures were grown in 125 cc. Erlenmeyer flasks, 40 cc. of the medium being placed in each flask. In order to keep a constant supply new cultures were made about every 2 weeks. Since *Phyllosticta solitaria* is a slow-growing fungus it took from 2 to 4 weeks for pycnidia with viable spores to develop. The cultures usually were above 4 weeks old (3 weeks in the case of culture No. 24).

By means of a sterilized needle pycnidia were transferred to a drop of distilled water. These were crushed, allowing the spores to escape into the drop, care being taken to have the spores evenly distributed throughout the liquid, and to have approximately the same number of spores on each slide. In the study of temperature relations spores were at first put to germinate in small drops of liquid on slides, but this method proved unsatisfactory, since the liquid evaporated at high temperatures. Consequently, the paraffin-ring method, allowing the use of larger drops of liquid, was devised. The slides were cleaned carefully but not sterilized and no special care was taken to use perfectly sterile liquid. They were then placed in Petri dishes that had been lined with moist filter paper.

For spores secured from nature, essentially the same methods were used. Pycnidia with a little adherent bark were removed from twigs by means of a scalpel and placed on a slide. The bark was cut or pycnidia crushed so as to free the spores into the liquid and the latter were placed in liquid on paraffin-ringed slides.

For the study of the effect of various nutrients on spore germination such solutions as rain water, tap water, distilled water, potato-dextrose broth, apple-fruit juice, bark decoction, K.U. solution, and modified Coons' solution were tried. The apple juice was made by boiling 70 grams of ripe apple in 500 cc. distilled water, filtering, and sterilizing. Bark decoction (50 grams of bark of apple twigs to 1000 cc. distilled water heated, then allowed to stand over night, and filtered) also was sterilized. The K.U. solution was made in the same way as the K.U. agar mentioned above, omitting the agar. The modified Coons' solution consisted of KNO<sub>3</sub> 2.02 gm., KH<sub>2</sub>PO<sub>4</sub> 2.72 gm., MgSO<sub>4</sub> 1.25 gm., dextrose 10 gm., distilled water

<sup>2</sup> Standard potato-dextrose broth: potatoes 140 gm., dextrose 10 gm., water 1000 cc., the whole being diluted to 1/10 its original strength and 20 grams of agar added.

<sup>3</sup> K.U. agar: KNO<sub>3</sub> 0.5 gm., KH<sub>2</sub>PO<sub>4</sub> 1.25 gm., MgSO<sub>4</sub> 0.75 gm., dextrose 1 gm., agar 20 gm., H<sub>2</sub>O 1000 cc.

1000 cc. In these studies the spores were suspended in hanging drops of the solution in microslide cells. The slides were placed in Petri dishes with moist filter paper and kept at room temperature. All cover glasses, microcells, Petri dishes, and solutions were sterilized and every precaution was taken to prevent contamination.

#### EFFECT OF LIGHT AND DARK

Spores were sown in distilled water on slides in moist Petri dishes. One dish was then left in the light at room temperature and the other, wrapped with black paper, was placed in a drawer at room temperature. In agreement with Guba's results the writer found no significant difference between light and dark. Seventy-five per cent of the spores placed in the light germinated, while but 68 per cent of those in the dark germinated. Similar results obtained when the experiment was repeated.

#### EFFECT OF TEMPERATURE

For this study the Petri dishes containing the slides were placed in dark chambers (light and dark, as shown above, having no apparent effect on germination) in a large temperature tank modeled after that described by Livingston and Fawcett (7). After preliminary adjustment each chamber in this tank assumes a different temperature and the temperatures remain constant until the range of temperatures is changed.

For the temperature studies spores were put to germinate in distilled water. Preliminary trials of this and other liquids resulted in somewhat better germination in potato-dextrose broth. This medium, however, becomes readily contaminated, and the use of distilled water obviated the necessity of sterilization. Distilled water allows from 90 to 98 per cent germination.

The spores of the apple-blotch fungus germinate rather slowly. Lewis (6) has reported that they germinate in the laboratory in from 12-18 hours and Scott and Rorer (10) report that they germinate in 15 hours. In this present study spores were examined on the second day, since it was found that earlier examination gave much lower counts. In determining the percentages of germination shown in table 1, experiment 1, from 100 to 200 spores, usually 150, were counted in each case. For table 1, experiment 2, the counts were of from 50 to 100 spores, ordinarily 100, while for table 2 approximately 50 spores were counted each time. Besides percentage of germination the average length of germ tube was calculated in each case. Each germ tube was measured carefully by means of an eyepiece micrometer and the sum of germ-tube lengths of all germinated spores was divided by the number of spores counted. Average germ-tube length, calculated

in this way, is recommended by Brown (2) as a better measure of the rate of early growth of a fungus than percentage of germination.

In table 1, experiment 1, the results of 3 trials are recorded. In the first trial the spores were counted the second day, while in the other 2 trials a count was made the second and fifth days. At a temperature of 5°–6° C. and at 34°–36° C. there was, in most cases, a very low percentage of germination. Even at 12°–14° C. and 29°–31° C. there was a comparatively low germination. The optimum temperature was 21°–23° C. but germination at 25°–27° C. was nearly as good. In the second experiment, reported in table 1, a slightly different range of temperatures was employed. This table shows a low percentage of germination at 8°–10° C. and a still lower one at 37°–38° C. The optimum in this case occurs at about 21°–22° C., although the percentages also are quite high at 25°–27° C.

The data secured by measurement of germ-tube lengths are presented in table 1, on the right. The germ-tube lengths were measured in microns. There was no appreciable difference in width of germ tubes. The average germ-tube length was shorter at the extreme low and high temperatures where there was a lower percentage of germination. In table 1, experiment 1, 3 of the 5 counts made show the greatest germ-tube length at 21°–23° C. the other 2 showing better at 25°–27° C. Also, in table 1, experiment 2, the optimum growth is found at 21°–22° C. and in table 2 the best germ-tube-length average is at 23° C. It seems that, in general, the highest percentage of germination and greatest average germ-tube growth agree.

The lowest and highest temperatures used in this study, namely 5° and 39° C., are believed to be close to the minimum and maximum temperatures for germination. It will be seen, however, that no temperature used completely inhibited germination. The optimum temperature (of those employed) is 21°–23° C. The actual optimum may well be at 24° C., since this temperature was not tried. The discrepancy between the author's results and those of Guba (4) cannot well be explained. It may be stated, however, that Guba's experimental data are very meager. The results here reported more nearly agree with those of Gardner (3), who found the optimum for germination to lie between 24° and 27° C.

Apple blotch is a disease of the central United States, being less prevalent in the northern part of its range and absent from extreme northern localities. It was thought an explanation of this fact might be found in the temperature requirements for spore germination. From these results, however, it is clear that no such limiting temperature requirements exist. Mix (8) has shown that the temperature requirements for spore production in culture do not afford an explanation of the present distribution of the fungus. Perhaps a study of the effect of temperature on infection by

TABLE 1.—*Effect of temperature on germination of spores of Phyllosticta solitaria from culture*

FIRST EXPERIMENT						
Temperature	Trial number	Germination		Average length of germ tubes		
° C.		Per cent		Microns		
	After	48 hrs.	120 hrs.	48 hrs.	120 hrs.	
5-6	1	15		2.32		
	2	2	6	0.14	0.61	
	3	1	3	0.21	0.29	
12-14	1	37		8.37		
	2	10	43	1.27	9.27	
	3	9	22	1.01	2.66	
17-19	1	37		6.77		
	2	32	55	5.78	9.17	
	3	17	52	2.42	5.87	
21-23	1	84		22.18		
	2	49	70	9.15	9.36	
	3	44	62	6.49	5.52	
25-27	1	77		19.82		
	2	47	65	9.81	7.98	
	3	37	55	4.46	6.24	
29-31	1	46		9.92		
	2	13	25	2.09	3.30	
	3	15	29	2.11	3.36	
34-36	1	23		3.96		
	2	3	3	0.31	0.25	
	3	6	9	0.77	1.10	

SECOND EXPERIMENT							
Temperature	Trial number	Germination			Average length of germ tubes		
° C.		Per cent			Microns		
	After	48 hrs.	96 hrs.	120 hrs.	48 hrs.	96 hrs.	120 hrs.
8-10	1	10	18		0.72	3.05	
	2	35		40	6.63		9.58
15-17	1	48	67		9.69	14.10	
	2	60		72	12.59		13.65
21-22	1	82	86		18.74	16.99	
25-27	1	62	79		10.92	14.40	
29-31	1		55			7.50	
37-39	1	3	5		0.22	0.57	
	2	15		17	1.61		1.01

*Phyllosticta solitaria* will furnish the desired information. Pending such a study it is necessary to fall back on the explanations offered by Gardner (3): a shorter growing season in the north unfavorable to mycelial growth, insufficient time for invasion of twigs by the fungus from petiole lesions, or retarded growth of cankers and production of spores in the spring.

Until these further possibilities are eliminated it is well to keep in mind the opinion of Anderson (1) that apple blotch may ultimately become serious in northern localities.

#### COMPARISON OF SPORES FROM NATURE AND FROM CULTURE

Although Kohl (5) reports only one experiment, he seems to have experienced much difficulty in obtaining germination of spores formed in culture. He states "it is conceivable that the common lack of viability of spores in culture may be attributable in part to injury by drying, since it requires approximately 35 days for the spores to mature in tube cultures." Apparently, Kohl means that this injury results from the desiccation of pycnidia and spores in old cultures, but it should be noted that elsewhere in the same paper he reports the occurrence of spores in 20- to 22-day-old cultures.

Since both Mix (8) and the writer have found abundant sporulation in cultures that had not begun to dry, there seems to be no point to Kohl's suggestion. However, in view of his experience, it seemed desirable to compare the germination of spores from culture with those from nature. Spores from both sources, therefore, were put to germinate at the same time in the same temperature chambers. The same general trend of germination (Table 2) was found in this experiment as in previous ones: that is, there was a lower percentage of germination at 15°-16° C. of spores from nature, as well as those from culture, and the optimum was 21°-23° C. in both cases. Spores from nature, however, showed consistently lower germination than those from culture. The former were obtained from cankered twigs about the first of June, 1932. Two other comparisons of spores from nature and from culture were made. On May 9 spores obtained from cankers were put to germinate at room temperature along with spores from culture. The former gave 54-60 per cent germination as compared with

TABLE 2.—Effect of temperature on germination (in 48 hours) of spores of *Phyllosticta solitaria* from culture and nature

Temperature	From culture		From nature	
	Germination	Average length of germ tubes	Germination	Average length of germ tubes
° C.	Per cent	Microns	Per cent	Microns
15-16	17	1.90	4	0.65
17	59	13.04	...	...
18-19	68	17.90	11	0.79
23	84	20.03	15	1.73
25	60	10.99	12	0.70

80-90 per cent germination of the latter. Again, on June 2, 36.8 per cent of spores taken from pycnidia on cankered twigs germinated at room temperature, while those from culture gave 56.6 per cent germination.

It may be concluded that spores from culture germinate quite as well as those from nature. The consistently higher germination of spores from culture obtained in these experiments may perhaps be explained on the basis that these spores were physiologically mature, while those obtained from twig cankers were not. (A discussion of physiological maturity of spores will be found later in this paper.)

#### EFFECT OF VARIOUS NUTRIENTS

Table 3 shows the effects of various nutrients on the germination of spores of *Phyllosticta solitaria*. Of the solutions used bark decoction allowed the best germination. Potato-dextrose broth also gave fairly high germination. K. U. solution and tap water, compared with distilled water, showed an inhibitory effect. (Tap water in this laboratory frequently is chlorinated.) Coons' solution caused a slight increase over distilled water. In table 3, on the right, are recorded the germ-tube lengths for spores placed in the various solutions. Usually the best solution for germination also was best for germ-tube growth. Bark-decoction and potato-dextrose broth enhanced the latter.

#### EFFECT OF MATURITY OF SPORES

Guba (4) believes that, in addition to moisture, nutrition, and proper temperature, maturity of spores is necessary for germination, stating that spores from young spore-bearing cultures fail to germinate. He found that less than 1 per cent of the spores from 16-day-old cultures germinated; and, by further comparison of 32-day-old and 46-day-old cultures, he decided that as the pycnidia aged the percentage of germination increased. The highest germination occurred when spores were taken from 1- to 1½-month-old cultures. From April 15 to July 15, Guba made repeated attempts to germinate spores from pycnidia on leaves, fruit, and bark, but with very little success. Roberts (9) tested germination of spores from bark cankers at various dates, but the number of spores germinating was too small to allow conclusions as to date of maturity. He obtained germination in distilled water after May 23, 1914; on May 13, 1915, he found 10 per cent, and after May 24, 75 per cent had germinated.

Kohl (5) offers the same reason for the lack of viability of spores. He believes that in nature and in culture there is an interval between morphological and physiological maturity, and states, without reporting any experiments, that this interval could not be shortened in culture by artificial means. He reports that, in southern Indiana, spores in twig cankers are



TABLE 3.—*Effect of various nutrients on germination of spores of Phyllosticta solitaria from culture*

Medium	Trial number	Germination		Average length of germ tubes	
		Per cent		Microns	
	After	48 hrs.	96 hrs.	48 hrs.	96 hrs.
Distilled water	1		8		0.70
	2	35	62	5.88	10.27
	3	23	55	4.23	10.96
Potato-dextrose broth	1	32	89	3.92	58.61
	2	79	80	14.70	28.84
	3	53	68	5.56	17.09
Coons' solution	1	0	37	0.00	2.54
	2	21	29	1.03	2.24
	3	27	57	2.03	11.86
Rain water	1	0	2	0.00	0.03
	2	11	24	1.38	1.88
	3	11	30	1.93	4.13
Tap water	1	1	5	0.03	0.62
	2	10	12	0.71	1.29
	3	4	22	0.62	7.80
K. U. solution	1	0	1	0.00	0.03
	2	10	17	1.50	12.00
	3	4	6	0.34	0.30
Apple-fruit juice	1				
	2	11	29	1.17	3.84
	3	2	18	0.06	2.04
Apple-bark decoction	1				
	2	73	96	16.68	98.93
	3	63	92	6.25	31.44
Distilled water + apple-fruit tissue	1				
	2		2		0.19
	1				
Distilled water + apple bark	2		86		53.35
	3		80		38.37

ordinarily morphologically mature about April 1 and physiologically mature by May 1.

In the present investigation study was made of the time of maturation of spores in culture. Pycnidia, as soon as they appeared, which was usually when the cultures were about 4 weeks old, were examined for spores. When first examined they contained droplets of protoplasm and no spores. In nearly every case it took from 4 to 7 days longer for spores to appear. Frequently, from one flask to another, there occurred a slight variation in the maturity of spores and their capacity to germinate. For example, in one flask 30 per cent of the spores germinated, while spores taken from a dupli-

cate flask showed only 20 per cent germination (Table 4). After the spores had matured they were put to germinate in distilled water at room temperature. They were examined on the 2nd and 4th days. This process, involving several cultures, was repeated at frequent intervals to allow, where necessary, for further maturity of spores (Table 4). In nearly every case, spores only one day old gave a low percentage of germination. With the advancing age of the cultures there was an increase in the percentage germination, the time of maximum germination varying somewhat with different cultures. In culture 299 the highest percentage of germination was found when the spores were 19 and 22 days old. In culture 342 the maximum was on the 15th day and in the other cultures it occurred on the 11th day. It seems that a period of maturation is necessary for best spore germination, although some germination occurs as soon as the spores are formed. Thirty-day-old spores show low percentages of germination and a little later they may not be found in cultures. Mix (8) also records this autolysis of spores occurring in old cultures. Table 4 gives the germ-tube lengths and the results show that the optimum time for germination usually is the best for germ-tube growth.

It may be concluded that a period of maturation is necessary to the best germination of spores from culture, although their period is shorter than Kohl seems to have observed. The period between the first appearance of spores and their maximum germination is relatively short. Since spores develop in culture in about 4 weeks and the most profuse germination is approximately 2 weeks later, spores taken from 1½-month-old cultures give best results. This is in agreement with Guba's report.

A few observations were made as to time of maturity of spores in nature. Pycnidia from twig-cankers, February 5, showed very few spores and these failed to germinate. Of 46 pycnidia examined March 14, 35 bore no spores, 8 contained very few, and 3 were nearly filled with them. No germination was obtained. On May 9, of 45 pycnidia examined one contained no spores, 9 very few, 19 many, and 16 were full of them. Germination of a few spores occurred in 4 days, 54 per cent in 5 days, and over 60 per cent in 6 days.

In this connection some unpublished observations made in this laboratory by Mix may be cited. Pycnospores were found in bark cankers on February 15 and March 7 and a few spores germinated in distilled water. Spores from pycnidia on twigs were put to germinate, on May 24, by the slide and drop method. Of six slides examined after 18 hours, only one gave a percentage germination and that was 45 per cent. On June 5, the average germination of spores from twig-cankers was 9 per cent in contrast to 90 per cent for spores from culture. On July 18, abundant germination was obtained of spores from cankers.

TABLE 4.—*Effect of different ages on germination of spores of Phyllosticta solitaria from culture*

Time after appearance of spores	Culture number	Germination		Average length of germ-tubes	
		Per cent		Microns	
Days	After	48 hrs.	96 hrs.	48 hrs.	96 hrs.
1	24a	0	0	0.00	0.00
	342	2	4	0.28	0.35
	299		67		86.22
	24b		4		0.40
	24c	0	0	0.00	0.00
2	24a	26	30	2.32	3.22
3	342	10	12	0.93	1.37
	24c	0	2	0.00	0.08
4	24a	22	32	3.45	5.16
	24b		31		3.02
5	24a	30	42	3.81	4.34
	342	11	12	1.02	1.33
	299		88		36.44
7	24c		8		0.89
	24a	50	57	5.20	9.14
	342	12	14	1.15	1.14
	24c	30	34	1.73	1.88
8	299	93	93	34.28	31.80
	24b	53	59	9.64	12.54
11	24d	16	16	3.24	3.19
	342		34		4.48
	299		95		36.05
	24b		63		12.76
	24c		36		2.50
15	24d		22		2.22
	342	35	40	5.34	5.10
	299	95	97	35.96	36.74
	24b	22	22	1.42	1.99
	24c		22		1.23
17	299	97		25.73	
	24b	22		1.83	
19	24d	53	62	6.51	8.57
	342		10		1.34
	299		98		32.98
	24c	16	16	1.13	1.20
22	299	97	98	38.07	38.78
	24b	9	9	1.41	1.08
	24c	16	16	0.96	1.12
25	24d	60	69	8.38	9.61
	342		6		1.09
	24c		9		1.04
29	342		4		0.43
	24b		8		0.77

Crosier, also working in this laboratory, found a few spores present in twig cankers on February 10, and obtained 13 per cent germination in bark decoction and 3 per cent in tap water in 24 hours.

The above data and the observations of other workers here cited indicate a longer interval between morphological and physiological maturity for spores from twig-cankers than for spores from culture. It is possible that a closer agreement might be found between spores formed in fruit blotches and those formed in culture.

#### EFFECT OF VOLATILE SUBSTANCES ARISING FROM PLANT TISSUES

The effect of various plant tissues on germination when placed near the hanging drop was observed. Apple-fruit tissue, apple bark, elm bark, pear bark, potato tuber, orange pulp, orange rind, onion scale, and onion leaf were used. Small pieces (approximately  $1 \times 6 \times 6.5$  mm.) and the larger pieces ( $1 \times 9 \times 10$  mm.), of each tissue were placed in the bottom of the cell, a hanging drop of distilled water containing spores being immediately above but not touching the plant tissue. The slides were placed in Petri-dish moist chambers. Spores were counted after 40 hours, since bacterial contamination could not be entirely prevented and counts made on the 3rd and 4th days would not have been reliable. In each case spores in distilled water without pieces of plant tissue served as a control.

The results of this study are shown in table 5. Bark tissue stimulates germination, apple bark being the most effective. On the other hand apple-fruit tissue, potato tuber, onion leaves and scales, and orange rind and pulp inhibit germination. Brown (2), in his studies on the germination of spores of *Botrytis cinerea* when exposed to volatile substances arising from plant tissues, found germination reduced or inhibited by potato tuber and onion leaves or bulb scales. He demonstrated that carbon dioxide was not the inhibiting factor. In the present investigation no experiments were attempted to find what might be the inhibiting or stimulating factor involved.

#### SUMMARY

Studies were made of the effect of various factors on the germination of spores of *Phyllosticta solitaria*.

Spores were taken, for the most part, from culture, suspended in distilled water, and germination counts were made at the end of 48 hours.

Light has no appreciable effect on germination.

The minimum temperature for germination is somewhat below  $5^{\circ}$  C., the maximum somewhat above  $39^{\circ}$  C., the optimum is close to  $23^{\circ}$  C.

Spores from pycnidia in bark cankers were found to have the same temperature requirements as spores from culture, but showed consistently lower germination. This may have been due to physiological immaturity of the spores from bark cankers.

TABLE 5.—*Effect of various plant tissues placed in the germination chamber on germination of spores of Phyllosticta solitaria from culture*

Tissue	Trial number	Germination		Average length of germ tubes	
		Per cent		Microns	
		small piece	large piece	small piece	large piece
Control—distilled water	1	12	12	0.86	0.86
	2	52	52	10.77	10.77
Apple-fruit tissue	1	0	0	0.00	0.00
	2	23	13	2.55	1.58
Apple bark	1	11	26	0.64	2.34
	2	69	72	23.76	25.10
Elm bark	1	10	11	0.97	1.06
	2	35	59	7.35	10.18
Pear bark	1	11	25	1.10	1.86
	2	52	53	11.12	10.98
Potato-tuber tissue	1	0	2	0.00	0.04
	2	42	51	2.73	5.88
Orange-fruit tissue	1	0	2	0.00	0.05
	2	2	3	0.16	0.26
Orange rind	1	0	0	0.00	0.00
	2	6	7	1.68	1.12
Onion scale	1	0	0	0.00	0.00
	2	1	0	0.08	0.00
Onion leaf	1	0	0	0.00	0.00
	2	4	2	0.57	0.52

When, instead of percentage germination, average germ-tube length (taking into account both germinated and nongerminated spores) was taken as the measure of viability, the results agreed in nearly every case with those obtained by the former method.

The observed temperature requirements for spore germination of *Phyllosticta solitaria* do not help to explain the present distribution of the fungus in nature.

Spore germination was more profuse in apple-bark decoction and potato-dextrose broth than in distilled water. Coons' solution caused a slight increase; tap water, rain water, apple-fruit juice, and K. U. solution had an inhibitory effect.

Spores of *Phyllosticta solitaria* formed in culture are capable of immediate germination; but increasingly better germination occurs with age, the maximum occurring when the spores are ten days or two weeks old. There is thus a brief interval between morphological and physiological maturity of spores formed in culture.

Of various tissues placed in the hanging drop, bark tissue acted as a stimulus, apple bark being the most effective. Orange tissue (both rind and fruit), onion scale and leaf, and apple pulp were somewhat inhibitory. No attempt was made to identify the volatile inhibiting substances.

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# THE GLADIOLUS DRY ROT CAUSED BY *SCLEROTINIA GLADIOLI* (MASSEY) N. COMB.<sup>1, 2</sup>

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(Accepted for publication June 29, 1933)

This disease is of importance in the growing of gladioli in commercial plantations as well as in private gardens. It has received some attention from the pathological point of view and its causal fungus has been studied and described (1, 6). The prominent structure of the fungus is a minute sclerotium, and the failure to find any functional spores, either on diseased plants or in artificial cultures, made it necessary to designate it as one of the *Mycelia Sterilia* under the binomial *Sclerotium gladioli* Massey (6).

While working with several sclerotium-producing fungi, it was found that this fungus will develop fruiting bodies as the result of a sexual process. The microconidia noted by Massey, and an ascogonial system in receptive structures, comprise the sexual components that accomplish the formation of apothecia of the *Sclerotinia* type. The recognition of a perfect stage makes it possible to extend our knowledge of this pathogen, and the purpose of this paper is to propose a new combination for its designation and to record the emended diagnosis. A number of new susceptibles have been encountered in the course of this work. These will be mentioned, together with some historical facts relative to the disease and the pathogen.

## THE SEXUAL NATURE OF THE FUNGUS

The fact that fruiting bodies of this fungus develop as the result of a sexual process has already been reported in a brief preliminary paper (2). A description of the technique used to induce the development of apothecia, together with other facts relating to the sexual mechanism of this fungus, has been published in a separate paper (3). It may not be out of place, however, to refer to some of these points here.

The microconidia of *Sclerotinia gladioli* are similar in shape and manner of production to those that have been recorded in several species of *Sclero-*

<sup>1</sup> Contribution No. 123 from the Laboratories of Cryptogamic Botany, Harvard University.

<sup>2</sup> Part of this work is taken from a thesis submitted to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy and the rest was done during the tenure of a National Research Fellowship in the Biological Sciences at the Laboratories of Cryptogamic Botany, Harvard University.

<sup>3</sup> The generous assistance and stimulating encouragement of Professor H. H. Whetzel, Professor W. H. Weston, Jr., and Dr. D. H. Linder are gratefully acknowledged.

tinia, Botrytis, and Sclerotium. Their function hitherto has always been obscure, but this investigation established the fact that they are spermatia. The receptive structures containing the coiled ascogonial system arise from a stromatic layer formed on the surface of substrates rich in carbohydrates. The spermatization of these with microconidia from a compatible isolate of the fungus results in the development of apothecia. In the absence of spermatia, or if microconidia from the same or an incompatible isolate are used for spermatization, there may be some elongation of the receptive bodies but no development of apothecia. Each monomycelial or monoascosporic culture gives rise to both receptive bodies and microconidial sporodochia when subjected to favorable conditions, but such cultures are self-sterile, reciprocally intersterile with some isolates (incompatibles), and interfertile with others (compatibles). There exists, therefore, the phenomenon of monoecism, if one may use this term to designate the presence of both sexual organs on the same fungus thallus, with the separation of various isolates into 2 groups exhibiting reciprocal intragroup sterility, intergroup fertility, while each isolate itself is self-sterile.

#### THE DISEASE AND THE PATHOGEN

*Sclerotinia gladioli* is responsible for a necrotic disease, known as dry rot, of the gladiolus and certain allied plants. The outstanding symptom is premature death brought about by the invasion and decay of the subterranean organs, including the basal portion of the leaf sheaths, while on the corms and cormels characteristic lesions are produced, often accompanied by vascular necrosis and mummification. On the decayed parts of the leaf sheaths, on the corm scales, and sometimes in or on the corm lesions are formed minute sclerotia, which serve as organs of resistance. The stroma, referred to above, is formed within badly diseased corms.

The first work of any importance done on this disease was by Wallace,<sup>4</sup> who applied the name dry rot to one of the 4 diseases of gladiolus corms that he studied. He suspected that he was including 2 diseases under this name, and, judging from his descriptions and photographs, this was the case. In addition to the true dry rot, he apparently had material of a common but undescribed storage disease of the corms caused by *Botrytis* sp. His description of one of the fungi isolated from gladiolus corms from Germany and New York State is unquestionably that of *Sclerotinia gladioli*. Two years later, Fitzpatrick (4) restricted the use of the name dry rot to the disease as now recognized.

Even before 1909, however, this disease seems to have been seen, although not recognized as such, for Wallace thinks that this was probably

<sup>4</sup> Wallace, E. Some bulb rots of gladioli. Unpublished thesis. Cornell University, 1909.



the disease referred to by Robinson (8, p. 139) in 1883 in England. Yet Wallace and other investigators have overlooked an article in 1906 by Ritzema-Bos (7) in which he describes a disease occurring on gladiolus and montbretia plants in Sassenheim, Holland. It is quite clear that his material consisted of plants affected with dry rot.

These several investigators all observed the development of small sclerotia by the causal fungus and noted the absence of sporulation. Hence, in 1918, Massey (5) proposed that it be placed in the form genus *Sclerotium*, and subsequently the present writer (1) referred to it as *Sclerotium* sp. in 1926. In 1928 Massey (6) erected the binomial *Sclerotium gladioli* and, as he observed for the first time the presence of microconidia, he suggested that the fungus might be a *Sclerotinia* because of the structure of these bodies and of the sclerotium, even though his attempts to bring about the development of apothecia were unsuccessful. These microconidia were found by Massey in 25- to 40-day-old test-tube cultures, where they appeared as small white granules less than 1 mm. in diameter, buried in the medium at the back of the slant adjacent to the wall of the tube. The writer finds, however, that by using Petri-dish cultures composed of plant stems and potato-2 per cent dextrose agar it is now possible to obtain more numerous and larger microconidial sporodochia in 12 to 18 days.

All of the papers dealing with this disease hitherto have noted its occurrence on the several varieties of the large-flowering gladiolus included in hybrids of the species *G. primulinus*, *G. communis*, etc. In the course of the present investigation, however, certain additional susceptibles have been encountered among other genera of the Iridaceae. During a visit to Holland in 1928, an examination of crocus corms as they were brought in from the field revealed the presence of this disease in a great many cases. In the same locality, also, under greenhouse culture, it was found on plants of *Lapeirousia* (*Anomatheca*) *cruenta* Baker and in field culture on *Tritonia* (*Montbretia*) *crocosmaeflora* Lemoine. Rather severe infections were observed on freesia plants in several greenhouses in Long Island, New York, in 1929, and in shipments of corms of this plant from Southern Europe to Canada. In all of these instances, the causal fungus was isolated and its identity confirmed. In connection with the question of host ranges, it is of interest to note here that when several varieties of rhizomatous iris, tulip, hyacinth, and narcissus were planted by the writer in soil from which gladiolus plants exhibiting 100 per cent infection had just been harvested, these plants all grew well and never showed any sign of infection. These tests, although limited, were sufficiently conclusive to justify mentioning them here, for a knowledge of the range of resistance and susceptibility among the commoner bulbous and rhizomatous ornamental plants may be

of importance to the nurseryman and gardener in arranging the rotation of their plantings.

#### EMENDED DIAGNOSIS

The recognition as sexual elements of the microconidia and the receptive structures developed by the stroma, and the manipulation of these with resulting production of a fruiting body, now makes it possible to give a complete technical description of this fungus, as follows:

#### *Sclerotinia gladioli* (Massey) n. comb.

Synonymy—*Sclerotium gladioli* Massey L. M.

Phytopath. 18: 519–529. 1928.

Mycelium septate, inter- and intracellular, multinucleate; in culture white, aerial portions tipped with buff when old, emitting a musty odor.

Sclerotia black, 90–240 x 90–300  $\mu$ , average 191 x 164  $\mu$ , consisting of a well-defined and compact rind of thick-walled cells surrounding a thin-walled, pseudoparenchymatous medulla, the cells of which are filled with small globules of oil (Fig. 1, A).

Stromatic tissue formed on substrates rich in carbohydrates, at temperatures of 18° to 24° C. Usually firmly attached to the substrate. Black, varying in thickness from 80–500  $\mu$ , with a rind of loosely aggregated, closely septate, black, thick-walled hyphae, and a prosenchymatous, hyaline medulla (Fig. 1, A).

Receptive bodies formed from the stromatic layer, .8 to 1.9 mm. tall, depending on age, .4 to .8 mm. broad, columnar, often branched, tapering to a rounded apex or occasionally slightly capitate, light brown, pilose, and covered with a thin layer of a mucilaginous substance. These bodies possess an external region of loosely interwoven, thick-walled, septate hyphae, hyaline towards the apex, becoming progressively darker towards the base and merging with the black hyphae of the stromatic rind. Within this, a region of light brown, densely packed hyphae, somewhat interwoven but running longitudinally and giving rise to an apical tuft of thinner-walled, septate hyphae that arch inwards to form a depression at the center of the apex. In the center is found a column of hyaline, less compact tissue composed mainly of a sparsely-septate, intricately coiled, multinucleate ascogonial system, which is terminated at the apex by trichogynous hyphae with their tips clustered beneath the overarching apical hyphae. On fertilization these structures develop into apothecia (Fig. 1, A, B, D).

Microconidia globose, 1.2–1.8  $\mu$  in diameter, uninucleate, and incapable of germination, but functioning as spermatia. Produced in a sporodochium made up of closely septate hyphae that give rise to numerous clusters of verticillately branched conidiophores that end in tapering, elongate, slightly

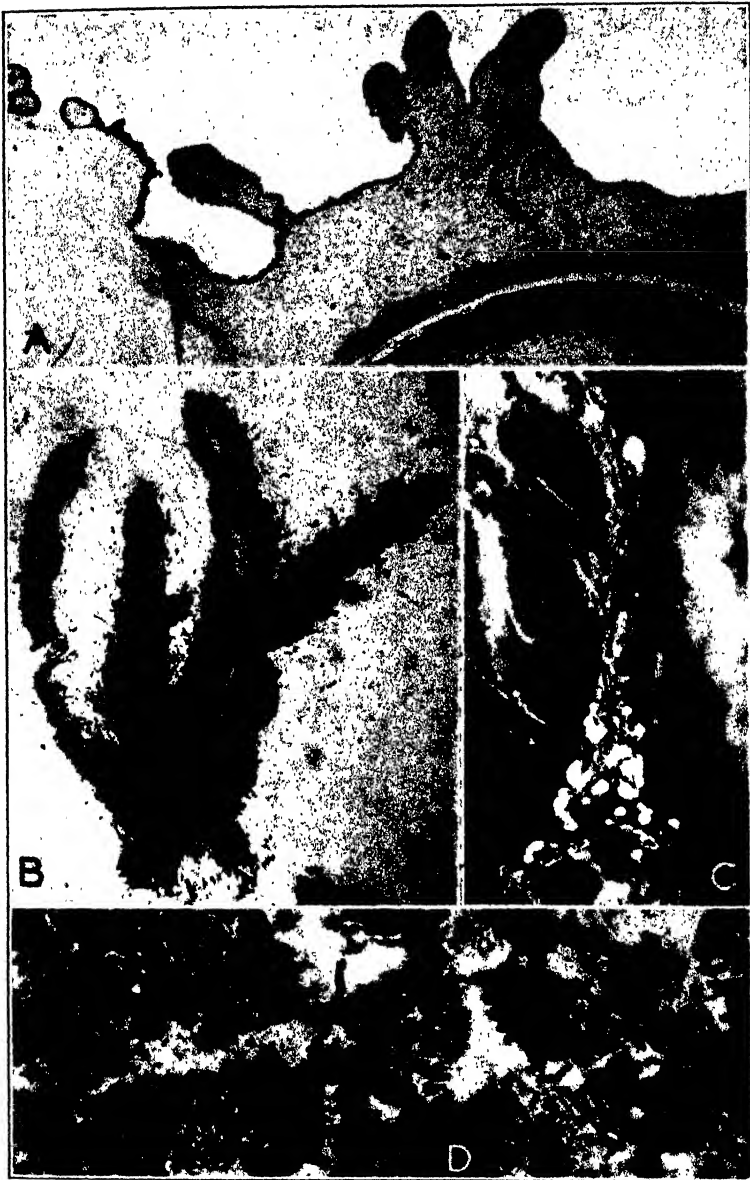


FIG. 1. A. Receptive bodies arising from the stroma formed on grain of wheat, a portion of which is evident at the lower right. Note the thin loosely constructed rind of the stroma as compared with the thicker and more compact rind of the three sclerotia at the upper left.  $\times 30$ . B. Branched receptive body showing the coiled ascogonial hyphae in the central core.  $\times 35$ . C. Microconidial sporodochia.  $\times 4.5$ . D. Part of a Petri-dish culture with receptive bodies.  $\times 4.5$ .

bent terminal cells on which the microconidia are developed successively in vast numbers embedded in a mucilaginous matrix, which, on drying, gives a waxy consistency to the whole sporodochium (Figs. 1, C and 2, C).

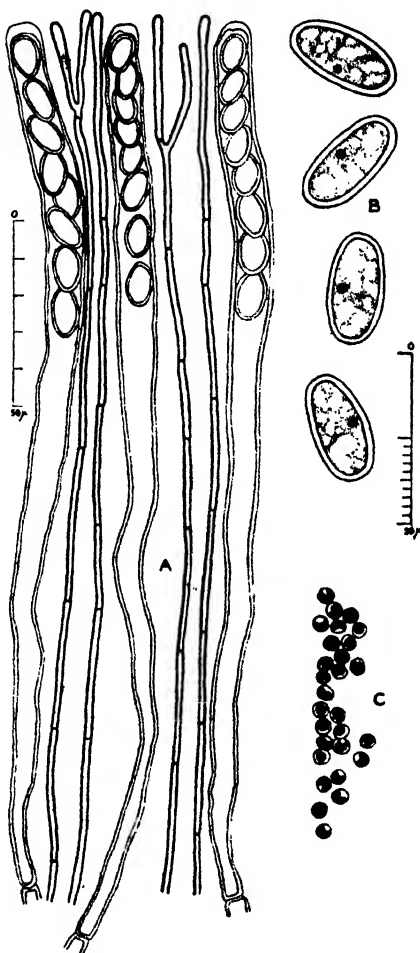


FIG. 2. A. Asci, ascospores, and paraphyses. B. Ascospores more highly magnified. C. Microconidia, magnified to the same extent as the ascospores.

Apothecia densely cespitose, stipitate, 3 to 7 mm. broad, 6 to 10 mm. high. Disc cinnamon brown, stipe chestnut brown (Ridgway). Hymenial surface umbilicate, convex-discoid. Margin strongly reflexed, deeply-crenate, sometimes entire or convolute. Lower surface tomentose, less densely so down the stipe. Context thick, prosenchymatous, infundibuliform, with a definite hypothecium (Fig. 3A, B, and C). Asci cylindrical to cylindro-clavate, opening by a pore,  $190.5\text{--}235.4 \times 8.5\text{--}9.2 \mu$ , average  $212.5 \times 9.06 \mu$ . Ascospores 8, unicellular, uniseriate, ellipsoidal, hyaline, uninucleate,  $10.2\text{--}$

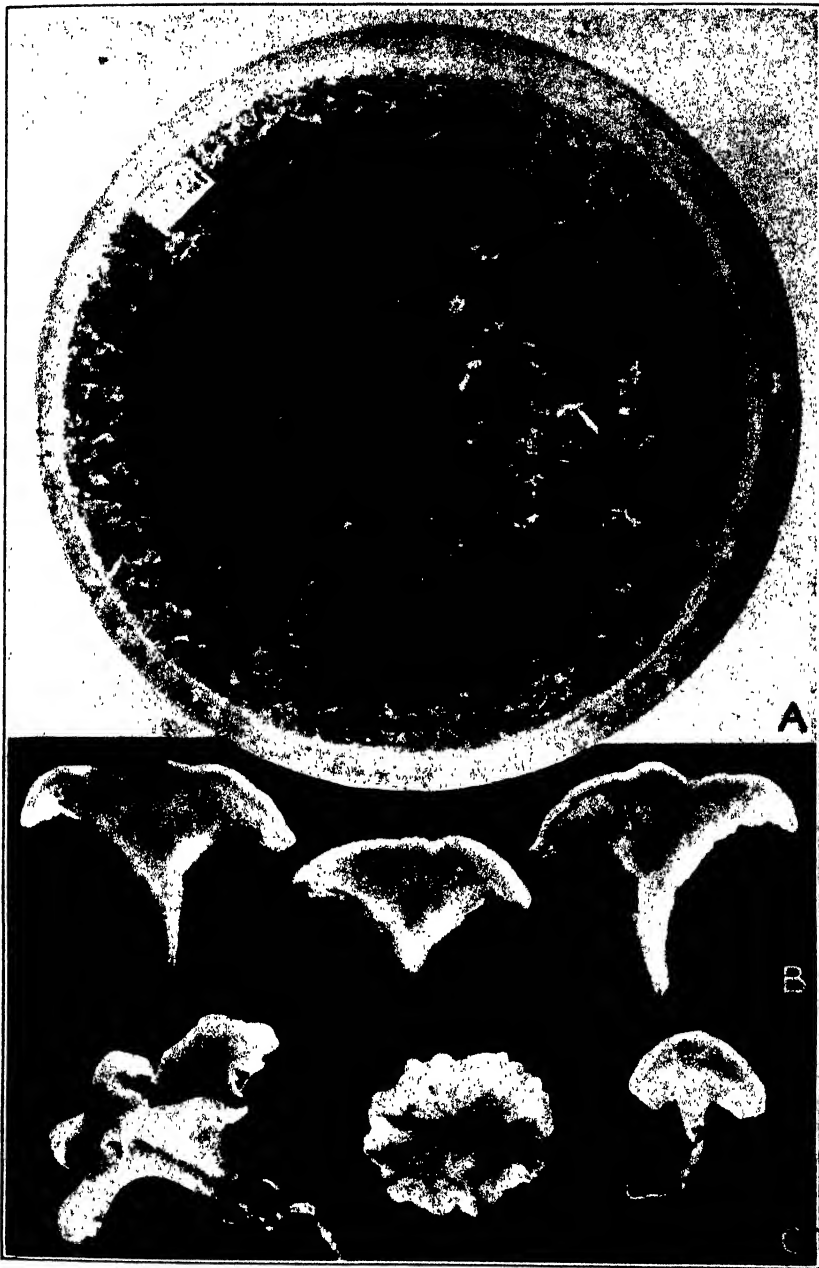


FIG. 3. A. Petri-dish culture bearing numerous apothecia.  $\times 8$ . B. Longitudinal sections of apothecia. Note hypothecium, thick medullary excipulum, and tomentose excipulum.  $\times 3.4$ . C. Three apothecia showing variation in shape.  $\times 4.2$ .

16.75 x 5.6–9.5  $\mu$ , average 14.04 x 7.25  $\mu$ , mode 13.5 x 7.25  $\mu$ . Paraphyses abundant, filiform to slightly clavate at apex, septate, hyaline 2.8–3.2  $\mu$  diam. (Fig. 2, A and B).

The cause of a necrotic disease known as dry rot, on the following suspects:—All cultivated varieties of *Gladiolus* spp., *Tritonia* (*Montbretia*) *crocosmaeflora* Lemoine, *Freesia* spp., *Lapeirousia* (*Anomatheca*) *cruenta* Baker, and *Crocus* spp.

Known distribution—United States, Canada, England, Scotland, Ireland, Holland, Germany, France, and New Zealand.

Type specimens of apothecia deposited in the Plant Pathology Herbarium, Cornell University, Ithaca, N. Y. No. 20136. Also in the Farlow Herbarium, Harvard University, Cambridge, Mass.

#### SUMMARY

The dry-rot disease of gladioli has been known for several years and its importance appreciated. The causal fungus apparently developed no functional spores and hence was named *Sclerotium gladioli* by Massey in 1928.

A sexual mechanism has now been discovered that involves the microconidia and the pilose receptive structures developed from a stroma. The sexual interaction of these yields apothecia of the *Sclerotinia* type. The life cycle of the fungus is, therefore, now completely known and its taxonomic relationship can be established.

The historical events of importance in connection with this disease and its pathogen are given.

The resistance and susceptibility of several common ornamental plants are mentioned and a member of new suspects are recorded.

The new combination *Sclerotinia gladioli* (Massey) is proposed and a detailed emended diagnosis is given.

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# HOST SPECIALIZATION OF PUCCINIA SORGHI

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(Accepted for publication July 8, 1933)

Arthur (2) was the first to produce the aecial stage of the rust of maize, *Puccinia Sorghi* Schw. In Indiana he found aecia on *Oxalis cymosa* in close association with overwintered débris of maize bearing telia of the rust. Aeciospores sown on young plants of maize resulted in the production of uredinia. The following year the connection was verified by inoculating *Oxalis cymosa*, *O. corniculata*, *O. Ortgiesii*, *O. Bowiei*, and *O. sp.* with overwintered teliospores (Arthur, 4). Pycnia and aecia developed only on *O. cymosa*. Aeciospores from the infection on *Oxalis*, sown on young maize plants, produced uredinia. Arthur was not able to infect maize from germinating teliospores, as Kellerman (18) had reported. Kellerman's results (19) were probably due to a mixture of viable urediniospores with the teliospores used in his experiments. Urediniospores may survive the winter to some extent. The writer has been able to obtain germination of urediniospores in late March from maize leaves that had been partly protected in the field (through the winter) in shocks of maize.

Kellerman, later, (19) ~~also~~ procured aecia on *Oxalis* from teliospores. In Iowa, Smith (25) obtained aecia on *O. corniculata*, *O. europea*, and *O. tropaeoloides*. Only pycnia were produced on *O. valdiviensis*. Negative results occurred with *O. cernua* and *O. rubra*. Miss Rice (24) obtained aecia on "*Xanthoxalis stricta* (L.) Small" and "*X. corniculata* (L.) Small" from inoculations with teliospores collected at Ann Arbor, Michigan.

In South Africa, Mary Pole Evans (16) found that *Oxalis corniculata* was a susceptible host for the aecial stage. *O. tenuifolia*, *O. livida*, *O. lateriflora*, *O. variabilis*, *O. balsamifera*, *O. monophylla*, and *O. hirta* were immune. In Austria, Hecke (17) inoculated a series of species of *Oxalis* with teliospores of *Puccinia Sorghi*. He obtained an abundant development of aecia on *O. stricta*. *Oxalis trapaeoloides* was less susceptible, and the aecia took six days longer to develop. Only pycnia were produced on *O. rosea*. Only flecks showed on *O. valdiviana*; *O. Bowiei* gave negative results.

<sup>1</sup> The investigations upon which this study is based were made while the writer was a member of the staff of the Botanical Department of the Purdue University Agricultural Experiment Station and of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 410.

Tranzschel (27) produced aecia on *O. corniculata* from telia collected in Transcaucasia.

Long (20) found aecia on *Oxalis corniculata* closely associated with rust on *Andropogon furcatus* at Denton, Texas. Telia were used to inoculate *O. corniculata*, and infection was obtained, but the plants were accidentally killed before aecia developed. Arthur and Fromme (8, p. 278) have assigned this rust to *Puccinia Sorghi*.

According to Arthur and Fromme, aecia of *Puccinia Sorghi* have been collected in North America on *Ionoxalis violacea* (L.) Small (*Oxalis violacea* L.), *Xanthoxalis filipes* Small (*Oxalis filipes* Small), *X. Langloisii* Small, and *X. stricta* (L.) Small (*Oxalis stricta* L., *O. corniculata stricta* Sav., *O. cymosa* Small). In their monograph the Sydows (26) list *Oxalis Bowiei*, *O. corniculata*, *O. filipes*, *O. Langloisii*, *O. obtusa*, *O. rosea*, *O. stricta*, and *O. violacea* as hosts.

Cummins (12) reports studies from which he concludes that *Puccinia Sorghi* is heterothallic. He obtained formation of aecia in 75 per cent of isolated infections treated with pycnial exudate from other infections. Of those left untreated only 14.3 per cent formed aecia.

The identities of some of the species of *Oxalis* are somewhat difficult to determine. Wiegand (28) has discussed the confusion that exists among the yellow-flowered species. He places *Xanthoxalis filipes* under *Oxalis filipes*, *X. Langloisii* under *O. corniculata*, *X. stricta* under *O. stricta*, and *O. corniculata stricta* and *O. cymosa* under *O. europea*.

For these investigations seeds of a number of species of *Oxalis* were obtained from various botanical gardens. Several of the local species were sent to Professor Wiegand for identification, and his determinations have been followed in naming the plants of *Oxalis corniculata*, *O. europea*, and *O. stricta*.

During the spring of 1928 a number of plants of *Oxalis corniculata* were infected in a manner similar to that used by Craigie (9, 10, 11). Leaves of maize bearing germinable teliospores were placed at different heights over plants of *Oxalis* in a moist chamber and were left for various periods until a combination was found that gave such a scattering of basidiospores that a large number of monosporous infections resulted. After inoculation the plants were placed under muslin cages to protect them against insects and were watered from below to prevent the spread of pycniospores by spattering. As soon as the first signs of infection (indicated by slight flecks) were noted all leaves bearing more than one infection were removed. With a little experience it was not difficult to detect infections occurring close together.

After the isolated spots had produced pycnia and after pycniospores



were exuding, a mixture of the exudate from a number of such spots was made. This was transferred to a series of isolated groups of pycnia. Another series was left untreated. In the one treated with the mixed exudate 260 out of 290 monosporous infections produced aecia. Of the 342 in the untreated series, only 29 produced aecia and 313 remained undeveloped.

Aecia were produced even if the transfer of pycniospores was delayed for some time. Thus in a series of plants of *Oxalis* inoculated on February 20, 71 isolated infections were treated with mixed exudate on March 4 and 147 were left untreated. In the treated group aecia were produced by 69 infections and in the untreated by only 11. Of those that did not produce aecia, 86 were treated with mixed exudate on March 25, 79 of which developed aecia. Thus the thalli were still in a condition favorable for diploidization 34 days after infection. These results agree with those reported by Cummins (12). They indicate that *Puccinia sorghi* is self-sterile.

From 1925 to 1928 an attempt was made to throw additional light on the host range of the aecial stage. The results are given in the accompanying table. In addition to testing the species of *Oxalis* listed in the table several collections of telia were sown on *Baptisia australis* (L.) R. Br., *Polygala Senega* L., *Pentstemon laevigatus* Ait., *P. secundiflorus* Benth., and *Viola papilionacea* Pursh, with negative results.

*Oxalis corniculata* was found to be the most favorable host, being susceptible to all collections of telia and producing an abundance of aecia. *O. stricta* gave mostly negative results; occasionally pycnia, and in one instance a few aecia, were produced. *Oxalis cernua* gave somewhat similar results. *O. bipartita* produced pycnia accompanied by necrosis in one case; the other results were negative. With the exception of *O. europea*, all the other species of *Oxalis* gave negative results. *O. europea* presented an interesting situation. To collections 2 and 5 it was susceptible, and abundant aecia<sup>2</sup> were produced. To the other collections it was resistant, at the most producing only a few pycnia.

It is evident that *Puccinia Sorghi* can be divided into at least 2 races: one producing only a few pycnia on *Oxalis europea*; the other, abundant aecia. It may be that Arthur (4) dealt with the latter, since he obtained aecia on *O. cymosa* (= *O. europea*, according to Wiegand). However, Arthur was not able to infect *O. corniculata*, whereas in this study both the collections of telia which infected *O. europea* also infected *O. corniculata*. These collections may, however, have been mixtures of two races: one producing aecia on *O. corniculata*, not on *O. europea*; the other, aecia on

<sup>2</sup> Collection 5 was received from M. A. Smith, and the results obtained agree with those reported by him (25).

*O. europea*, not on *O. corniculata*. If they are not mixtures, the existence of a third race is indicated, distinguished by having both *O. corniculata* and *O. europea* as susceptible species. Still another race may be indicated by the results of Hecke (17) and Rice (24), who obtained aecia on *O. stricta*. This species of *Oxalis*, as interpreted by Wiegand, was resistant to all telial collections used in this study. Aecia collected on *O. filipes* and *O. violacea* have also been assigned (Arthur and Fromme, 8) to *Puccinia Sorghi*. Neither of these species proved to be susceptible in these investigations.

TABLE 1.—Relative susceptibility of species of *Oxalis* to *Puccinia Sorghi*

Species inoculated	Reaction to collections of telia <sup>a</sup>							
	1 <sup>b</sup>	2	3	4	5	6	7	8
<i>Oxalis articulata</i> Sav. . . . .						—	—	
“ <i>bipartita</i> A. St. Hil. . . . .			—		O	—		
“ <i>brasiliensis</i> Lodd. . . . .	—	—			—	—	—	
“ <i>carnosa</i> Mol. . . . .	—	—			—	—	—	
“ <i>cernua</i> Thunb. . . . .	—	—			O(I)	—	—	
“ <i>corniculata</i> L. . . . .	OI	OI	OI	OI	OI	OI	OI	OI
“ <i>crassipes</i> Urb. . . . .	—	—			—	—	—	
“ <i>Deppei</i> Lodd. . . . .	—	—	—		—	—		
“ <i>europea</i> Jord. . . . .	—	OI	—(O)	—(O)	OI	—	—	—
“ <i>filipes</i> Small . . . . .						—	—	
“ <i>floribunda</i> Lehm. . . . .						—	—	
“ <i>lasiandra</i> Zucc. . . . .	—	—	—		—	—		
“ <i>latifolia</i> H. B. & K. . . . .	—	—	—		—	—	—	
“ <i>stricta</i> L. . . . .	—	—	—O		—	—	—	O(1)
“ <i>valdiviensis</i> Barn. . . . .		—			O	—	—	
“ <i>violacea</i> L. . . . .	—	—	—		—	—	—	

<sup>a</sup> — = negative results

O = pycnia produced in abundance

(O) = few pycnia

I = aecia produced in abundance

<sup>c</sup> (I) = few aecia

<sup>b</sup> Collections of telia were made as follows: 1–2, at La Fayette, Indiana (studied in 1925); 3–4, at La Fayette (studied in 1926); 5, in Decatur Co., Iowa, by M. A. Smith (studied in 1926); 6–7, at La Fayette (studied in 1927); and 8, at La Fayette (studied in 1928).

As previously shown (Mains, 22, 23), several physiologic forms may be distinguished in *Puccinia Sorghi* by the reactions of a selected set of inbred lines of maize. The relationship of these physiologic forms to the races differentiated by *Oxalis europea* has not been thoroughly established. Of those collections to which *O. europea* was resistant, collection 3<sup>c</sup> proved to

<sup>c</sup> Aeciospores produced by the infection of *Oxalis* were used to inoculate a series of differential strains of maize.

be physiologic form 1; collection 1, a mixture of physiologic forms 1 and 3; and collections 6 and 8, physiologic form 4. Collection 2, to which *O. europea* was susceptible, proved to be physiologic form 3. These results do not indicate any correlation, but they are not extensive enough to justify conclusions.

In 1920 aecia produced on *Oxalis corniculata* were used to inoculate seedlings of *Andropogon furcatus*, *A. scoparius*, and *Zea mays* (Golden Bantam). Only maize developed uredinia. In 1921 aecia from *Oxalis corniculata* were used to inoculate seedlings of *Andropogon furcatus* Muhl., *A. scoparius* Michx., *Holcus Sorghum* L., *Tripsacum dactyloides* L., *Sorghastrum nutans* (L.) Nash, *Coix lachryma-jobi* L., *Euchlaena mexicana* Schrad. (*Florida teosinte*), and *Zea mays* (Golden Bantam). Only teosinte and maize showed infection, both developing abundant uredinia.

The results obtained from aecia were supported by those secured from uredinia. Rust collected on maize at La Fayette, Indiana, in 1920 and sown on seedlings of *Andropogon furcatus*, *A. scoparius*, and *Zea mays* resulted in the development of uredinia on maize only. Likewise, in 1922, inoculations with urediniospores collected at La Fayette, Indiana, produced uredinia on maize. *A. furcatus*, *A. scoparius*, *Erianthus divaricatus* (L.) Hitchc., *Coix lachryma-jobi*, and *Tripsacum dactyloides* gave negative results. These results agree with those of Kellerman (18), who was able to infect teosinte "*Euchlaena luxurians*," but not *Sorghum vulgare*, *Saccharum officinarum*, or *Tripsacum dactyloides*.

Arthur and Fromme (8) list *Andropogon furcatus* as a host for *Puccinia Sorghi*. The results obtained in these investigations indicate that the rust on maize probably does not infect *Andropogon* and is at least a distinct race. It seems doubtful whether the rust on *A. furcatus* belongs in *Puccinia Sorghi*. This disposition of it was apparently based on cultures by Long (20), who showed that rust on *A. furcatus* produced aecia on *Oxalis corniculata*. Previous investigations have shown that rust on *Andropogon* (*Puccinia Ellisiana*, *P. Andropogonis*) produces aecia on species of *Viola* (Arthur, 7; Long, 20, 21), *Pentstemon* (Arthur, 1, 5, 6; Long, 21), and *Comandra* (Arthur, 3, 4). Davis (13, 14, 15) has found that *P. Andropogonis* also develops aecia on species of *Polygala*, *Lupinus*, *Amphicarpa*, and *Xanthoxylum*. Apparently *P. Andropogonis* contains a number of races differing in their aecial hosts. It would seem best, therefore, to consider the *Andropogon-Oxalis* rust another race of *P. Andropogonis*, rather than a race of *P. Sorghi*.

#### SUMMARY

1. Additional evidence is given that *Puccinia Sorghi* is self-sterile. Aecia do not usually develop from infections from single basidiospores.

2. Pycnia and aecia were produced on *Oxalis corniculata* and *O. europea*. Pycnia and occasionally a few aecia developed on *O. cernua* and *O. stricta*. Pycnia rarely developed on *O. bipartita* and *O. valdiviensis*. Negative results were obtained with *O. articulata*, *O. brasiliensis*, *O. carnosa*, *O. crassipes*, *O. Deppei*, *O. filipes*, *O. floribunda*, *O. lasiandra*, *O. latifolia*, and *O. violacea*.

3. *Oxalis corniculata* was very susceptible to all the collections of the rust that were studied. Aecia were obtained on *O. europea* with only 2 out of the 8 collections. This indicates that there are 2 races of *Puccinia Sorghi* that may be distinguished by the susceptibility of *O. europea*.

4. Since aecia have been collected on *Oxalis filipes*, *O. stricta*, and *O. violacea*, the marked resistance of these species noted in these investigations indicates other races of *Puccinia Sorghi*.

5. Inoculations from rust derived from maize (aecia and uredinia) produced uredinia on maize and teosinte. Negative results were obtained with *Andropogon furcatus*, *A. scoparius*, *Coix lachryma-jobi*, *Erianthus divaricatus*, *Holcus Sorghum*, *Tripsacum dactyloides*, and *Sorghastrum nutans*.

6. It is suggested that the *Andropogon* rust with aecia on species of *Oxalis*, which has been placed in *Puccinia Sorghi*, should be considered a race of *Puccinia Andropogonis*.

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# A PHYTOPHTHORA DISEASE OF SNAPDRAGONS

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In March, 1932, a wilt of snapdragons was found in several greenhouses near San Leandro, California. The disease was first found in the flats into which the young plants were transplanted from the seed bed. When the grower further transplanted the snapdragon plants to the large beds in the greenhouse, he selected apparently healthy specimens from flats on which some damage had been done by the disease. Later the disease appeared in the large greenhouse beds, and eventually 50 per cent of the plants were affected. Injury ranged from killing soon after transplanting to wilting when nearly ready to produce flowers. The grower had not experienced the trouble prior to this time and was unable to discover the source of the disease. A number of the affected plants were sent by him to the plant pathological laboratory of the California State Department of Agriculture, where the cause of the wilt was determined.

## SYMPTOMS OF THE DISEASE

Wilting of the plants was brought about by a girdling of the stems at the ground line or slightly above it. A slightly yellow water-soaked area appeared on the healthy white stem tissue. As this lesion increased in size the older portions of the lesion became yellow, then brown, and finally almost black. The lesion usually enlarged until it girdled the plant and extended up and down the stem for several inches. In the last stages of the disease the outer portion of the stem sloughed away, exposing the hard woody xylem tissue.

Plants when first attacked wilted slightly during the middle of the day and recovered at night. In 2 or 3 days the most severely affected plants wilted permanently and later became dry and brown. Occasionally, plants existed in a semiwilted condition for several days or weeks. Others did not become completely girdled and did not wilt. In damp portions of the greenhouse where the stems of the plants were wet, lesions were seen that extended up the main stem and onto the side branches.

## VARIETAL SUSCEPTIBILITY

Three varieties of snapdragons were being grown in the greenhouses, all of which were susceptible to the disease, but there was considerable variation in the varieties as to the degree of susceptibility. The variety Cheviot Maid was most resistant to the disease, only 20 per cent of the

plants being killed. Thirty per cent of the variety Jenny Schneider were wilting, and more than 75 per cent of the variety Roman Gold were destroyed by the disease. The 3 varieties were not grown in separate parts of the greenhouse but were mixed indiscriminately through the beds.

#### ORGANISMS ASSOCIATED WITH THE DISEASE

Cultures were made from a number of diseased plants found in the grower's greenhouse and 3 organisms were consistently isolated. One was a bacterium. Another was a fungus, later identified as *Cephalosporium acremonium* Corda. The third organism, also a fungus, was a species of *Phytophthora*. These appeared repeatedly in the plates, either as pure cultures or as mixed cultures. Each organism was grown in pure culture and used in an inoculation study. The results of this study (Table 1) show conclusively that the species of *Phytophthora* was the organism causing the disease and the other two were secondary invaders.

TABLE 1.—*Infection results obtained by inoculating snapdragon plants with Phytophthora, Cephalosporium, and bacteria singly and in combination*

Type of inoculum placed around stems of the plants	No. of plants inoculated	No. of plants diseased
Phytophthora on corn-meal-sand medium	5	5
Cephalosporium on corn-meal-sand medium	5	0
Bacterial spore suspension	5	0
Phytophthora and Cephalosporium each on corn-meal-sand medium	5	5
Phytophthora on corn-meal-sand medium and bacterial spore suspension	5	5
Cephalosporium on corn-meal-sand medium and bacterial spore suspension	5	0
Phytophthora and Cephalosporium each on corn-meal-sand medium and bacterial spore suspension	5	5
Check plants	5	0
Total	40	20

In all cases where *Phytophthora* caused plants to wilt it was recovered in pure or mixed cultures. There seemed to be no perceptible degree of difference in pathogenicity where the *Phytophthora* was used in pure culture or in association with the other 2 organisms.

After it had been determined that the *Phytophthora* was the pathogenic organism, 2 additional groups of snapdragon plants were exposed to cultures of the fungus grown on corn-meal-sand medium. The inoculum was placed around the stem at the soil line and kept moist. Out of 55 plants

thus treated all were found to be diseased by the *Phytophthora* fungus. Fifteen check plants remained in a healthy condition. On the plants that were affected after exposure to the fungus, the symptoms of the disease were identical with those first observed in the greenhouse at San Leandro. Some of the plants began to wilt about 4 days after being exposed to the fungus growing on the corn-meal-sand medium. Other plants remained free of any wilting for as long as 2 weeks after being exposed. Lesions ranged from a light yellow or light grayish, water-soaked area to a dark brown, soft mass. On all of the plants the cortex was badly decayed and slipped off the woody xylem core of the stem very easily.

#### DESCRIPTION OF THE CAUSAL FUNGUS

The mycelium is a long, very irregularly branched tube showing a few cross walls when old. On artificial media the mycelium presents a warty appearance. The hyphae are very granular and slightly yellow. Within the host tissue the mycelium is not so irregularly shaped as it is on artificial media. The hyphae range in diameter from 4 to 15  $\mu$ . On potato-dextrose agar the mycelium is dense, with an abundant aerial growth. On corn-meal agar the growth is very dense with much aerial growth. On oatmeal agar there is practically no aerial mycelium but there is a heavy growth on the surface and within the agar.

Conidia are fairly abundant on both potato-dextrose and corn-meal culture media. In size, they measure 20 to 25 by 30 to 45  $\mu$ . They are ovate in form, and each shows a distinct papillum. In color they are a light yellow, with a granular content. The conidia are terminal, being borne on slender hyphae branching off the larger mycelium.

Occasionally, the conidia have been observed germinating. Usually the germ tube arises near the apex of the conidium. A number of conidia have been observed producing swarm spores. The spores began moving within the sporangium and were extremely active for a half hour. Following this, a vesicle was seen to form near the papillum and the swarm spores swam into it. The vesicle was ruptured in a very short time and the spores swam free in large numbers.

Antheridia and oogonia were present both in cultural media and within the host tissues. The antheridium was roughly semispherical in form and lodged against the side of the spherical oogonium. The antheridium was not located at the base of the oogonium, and no germ tube was observed entering the oogonium. Oospores were numerous in the decayed tissue of the host plant. Apparently they form after the tissues are in an advanced stage of deterioration. In newly invaded tissues the mycelium can be seen in the cells. As the decay progresses the cells of the host turn brown and



completely collapse into a soft mass. The mycelium cannot be seen in this brown mass, but the oospores appear at this stage of decay.

#### TAXONOMY OF THE CAUSAL ORGANISM

The morphology of the fungus, as described above, shows clearly that it is one of the Phytophthorae. After a study of the oogonia, oospores, and conidia was made, the fungus was closely compared with several well known species of the genus. *Phytophthora mexicana*, described by Hotson and Hartge (3), was found to differ from the snapdragon fungus in having longer and more narrow conidiophores. Also, *P. mexicana* is described as having the antheridia at the base of the oogonia, thus differing from the fungus described in this paper. *P. parasitica* var. *rhei*, described by Godfrey (2), produces haustoria, while no such structures were seen in the snapdragon tissues. The fungus on snapdragons was also found to differ from *P. syringae* in the size of conidiophores as described by Bruyn (1). Rosenbaum (4), in his studies of the genus Phytophthora, divides it into 3 groups, based on the position of the antheridium with reference to the oogonium. By this classification, the fungus found on the snapdragons belongs in the *cactorum* group. A comparison of the snapdragon fungus with various species of the *P. cactorum* group, as outlined by Rosenbaum, resulted in the conclusion that the Phytophthora isolated from the snapdragons is closely allied to if not identical with *Phytophthora cactorum* (Lebert & Cohn) Schröt. This conclusion is further strengthened by a study of a description of the species *P. cactorum* as given by Stevens and Plunkett (5).

#### HISTOLOGICAL STUDIES OF DISEASED HOST TISSUE

Sections of diseased snapdragon stems were fixed, sectioned, and stained for study. An examination of stained slides showed that when the fungus had thoroughly invaded the stem the outer tissues had collapsed entirely. The epidermis was the first tissue to be invaded, following which the fungus penetrated rapidly through the cortex and phloem tissue to the cambium. Eventually the fungus made its way into the xylem, especially along the medullary rays. As the lesion enlarged around the spot of initial infection, the fungus traveled first in the cambium layer. Following the invasion of the cambium cells, the fungus penetrated the phloem and cortical cells. As the fungus progressed in its growth all of the cells except those in the xylem area collapsed and formed a brown shapeless mass. Among these collapsed cells were many oospores. (Fig. 1, B.)

#### CONTROL MEASURES

In attempting to find a means of eliminating the trouble on the snapdragons, the greenhouse was examined for sources of infective material.

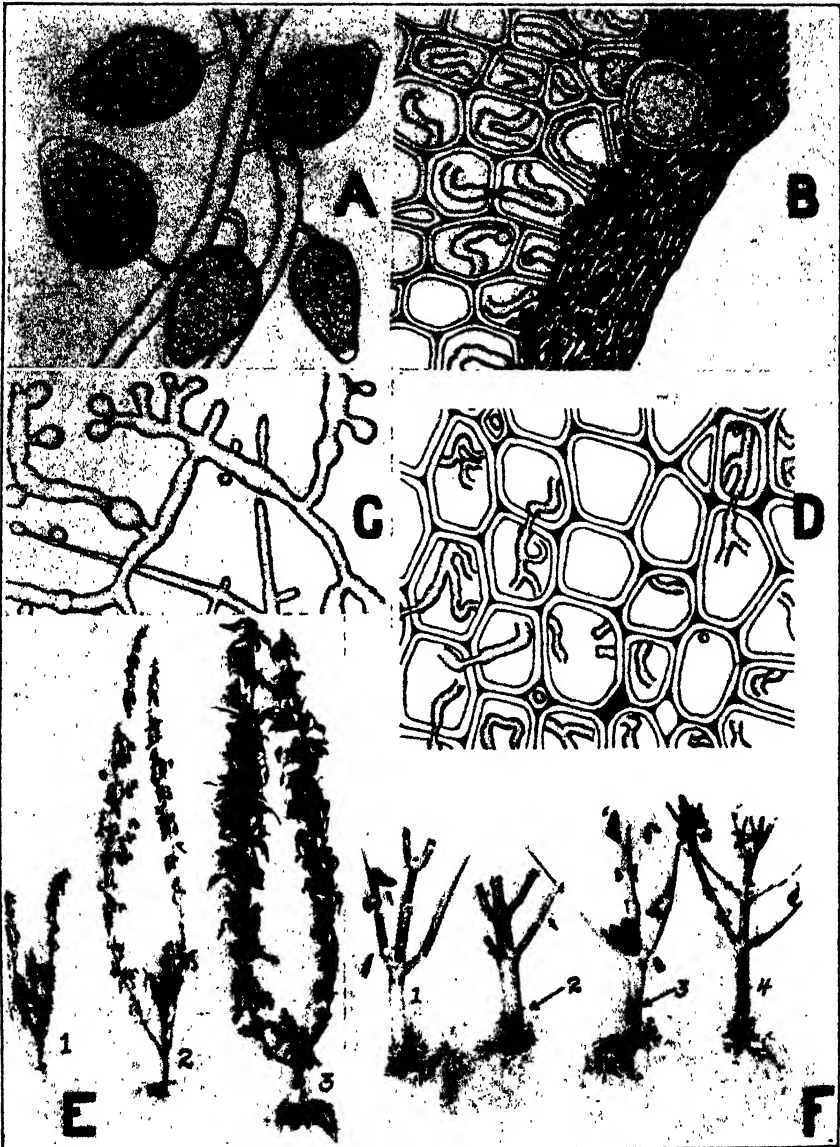


FIG. 1. A. Conidiophores of *Phytophthora cactorum* showing appearance of terminal papilla. B. Cross section of diseased snapdragon stem showing collapsed cortical tissue, an oogonium, and an antheridium. C. Mycelium grown on potato-dextrose agar culture. D. Cross section of xylem tissue showing mycelium within the cells. E. (1) Snapdragon plant stunted and killed by an attack of *P. cactorum*; (2) wilted plant attacked just before the blooming stage; (3) healthy plant. F. Roots and main stems of snapdragon plants: (1) healthy; (2) small lesion; (3) girdled by lesion; (4) cortical tissue sloughed off main stem, exposing woody xylem.

After a thorough search and tests of the soil and water used in the greenhouses, the parasite was located in the compost pile from which soil was taken to fill flats used at the time of transplanting the seedlings from the seed bed. Flats full of the compost soil were placed in a steam autoclave and subjected to a steam pressure of 10 pounds for half an hour. The soil was treated only once and was used for transplanting of plants from the seed bed as soon as it had cooled. This treatment completely killed the fungus, as shown by the fact that the disease did not affect plants grown in soil so treated and used in the transplanting flats. Soil believed to be free of the fungus was used to replace old soil in the large greenhouse beds. No disease appeared when plants from the steam treated flats were transplanted into the greenhouse.

#### SUMMARY

(1) A hitherto unreported wilt of snapdragons is described as to symptoms.

(2) Three organisms, an undetermined bacterium, *Cephalosporium acremonium* Corda, and a *Phytophthora* species were isolated from diseased snapdragon tissues. Inoculation experiments using the 3 organisms showed that the *Phytophthora* species was the pathogenic organism causing the disease.

(3) The fungus is described as to its morphology. A comparison with known *Phytophthora* species shows that the organism here described is closely allied to if not identical with *Phytophthora cactorum* (Lebert & Cohn) Schröt.

(4) A histological study of the fungus in the plant tissue is discussed.

(5) Control measures which have totally eliminated the disease from the affected greenhouses are given.

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# A METHOD OF RECORDING THE DISTRIBUTION OF COPPER DUSTS OR SPRAYS ON LEAVES

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When experiments were undertaken recently to determine more precisely some of the factors that increase the efficiency of potato spraying, it was evident at once that some more direct method than field tests was desirable with which to make preliminary studies. Questions such as these were being raised:—What pressure is most profitable? How many nozzles to the row are desirable? What kind of a spray boom is most efficient? How should it be adjusted? What kind of nozzles should be used? How much material should be applied? Similar questions are being asked in connection with dusting. If it were possible to spray or dust small areas of a field with each of several arrangements of nozzles and booms and then determine at once the kind of distribution obtained, progress in testing modifications of the spraying and dusting apparatus would be facilitated.

Working with this aim in view, there was developed a method of making prints on paper directly from the sprayed leaves to show the distribution of the copper. As a number of other workers have shown an interest in this method, it is now described in the hope that it may prove useful in similar cases.

The paper is prepared by dipping it in an acidifying solution of potassium ferrocyanide in water. The acid is added to render the copper of Bordeaux mixture soluble. The potassium ferrocyanide in the paper reacts to give the familiar brown precipitate (cupric ferrocyanide, Hatchett's brown) well-known to those who have used this reagent for testing Bordeaux mixtures for soluble copper. This precipitate remains adherent to the surface of the paper, where it produces a brown spot the size and shape of the Bordeaux spot on the leaf.

The concentration of the solution used apparently is not very critical. Two grams of potassium ferrocyanide and 5 cc. of acetic acid to 100 cc. of water were satisfactory. Nitric acid (2 cc.) may be used in place of the acetic acid.

Iron reacts with potassium ferrocyanide to give Prussian blue, so that this paper may also be used in tests for iron. For this reason iron must be avoided both in the paper and in the utensils used in the preparation of it. The vessels used to hold the solution should be glass or glazed earthenware. While extensive tests were not made, there were numerous disappointments at the beginning, apparently due to the presence of a small amount of iron

in the paper, especially in mimeograph paper. When tests were made later with a series of samples of different kinds of paper, bond papers were selected as most satisfactory.

After the paper has been thoroughly wetted with the solution, it may be kept moist until ready for use or, if dried, must be moistened again before it is used. It may be readily carried about and kept moist in paraffined envelopes, but here, again, metal fasteners must be avoided.

Samples of potato leaves may be collected to show the distribution of spray or dust on different parts of plants or on any particular part, as desired, and should be allowed to lose their turgescence before they are in best condition for printing. The moist paper is then placed on a cotton pad covered with muslin. The leaves or leaflets are arranged on the paper as desired with the upper side down. A second sheet of the prepared paper is placed on top of the leaves if a print of both sides of the leaves is to be made. Another cotton pad is then added. There are then in order a cotton pad, a prepared paper, a layer of leaves, another prepared paper and another cotton pad.

Immediately after this is done the whole pile is placed in a press. An old letter press is convenient for the purpose. Medium pressure at the beginning seems to avoid running together of the spots if the paper is too wet. Free drops of water on the paper are to be avoided for the same reason. Five to 10 minutes in the press is sufficient. The press should be tightened occasionally during this time.

After pressing, the paper is taken out and washed in water to remove the excess chemicals and thus avoid further reaction. It may then be dried to form a permanent record. The brown spots on the paper represent the Bordeaux spots on the leaf. One sheet records the spots on the upper side of the leaf, the other those on the lower side.

Any iron present, such as impurity in the copper sulphate, is recorded in blue. Incidentally, labels written on this paper, before washing, with ordinary fountain pen ink are changed to a permanent blue, which does not wash out.

Photographs of prints made from sprayed leaflets are shown in figure 1. Only a sample can be shown that was selected from a large number to represent the results obtained in comparing different pressures. The 3 prints of the upper row represent the upper side of the leaflets and those in the lower row the under side of the same leaflets. The first print at the left represents a sample taken from leaves sprayed at 400 pounds' pressure, the second, samples sprayed at 200 pounds' pressure but with nozzles with a larger orifice to apply as much material as at 400 pounds' pressure and the third shows a sample at 200 pounds' pressure with the same nozzle

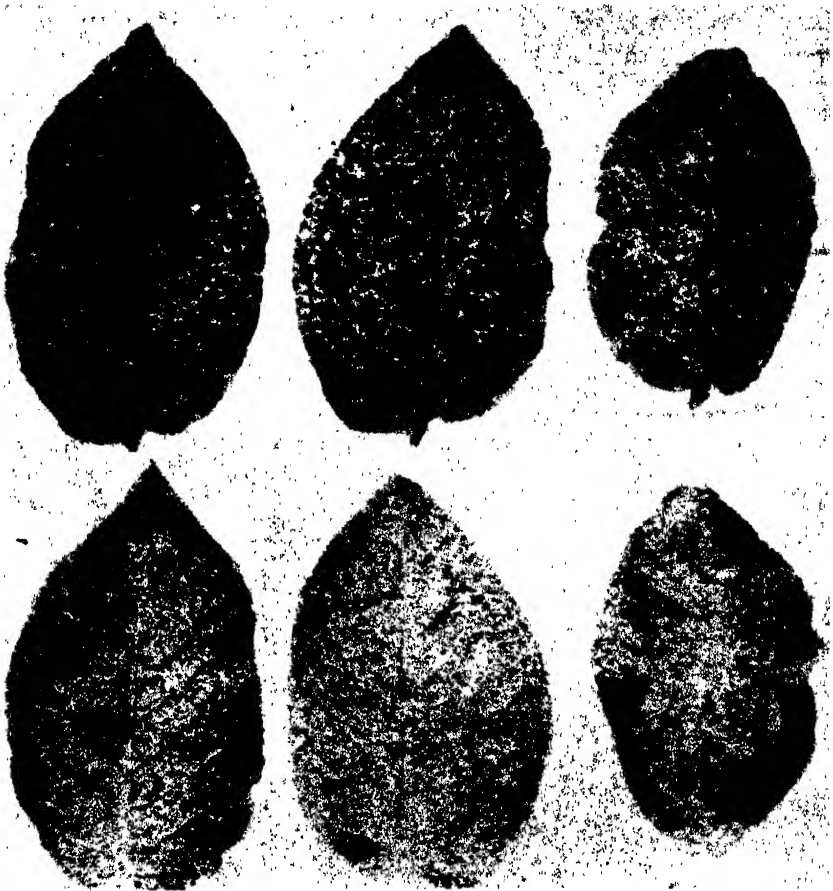


FIG. 1. Prints of leaflets sprayed with Bordeaux. Upper sides of the leaflets are shown in upper row, and under sides of the same leaflets in lower one. Beginning at the left, the first leaflet was sprayed at 400 pounds' pressure, the second at 200 pounds' pressure with the same amount of material as the first, and the third at 200 pounds' pressure with a smaller amount of material but with the same size nozzle orifice as the first.

orifice as at 400 pounds. The lower row shows the under sides of the leaflets in the same order.

It can be seen that the drops of Bordeaux have run together more when the larger amounts are applied at the lower pressure.

Some difficulty was encountered late in the season in trying to get good prints. This was due to the accumulations of copper on the leaves to such an extent that they were completely covered. To get good prints of any one spraying, it is, therefore, desirable to apply the tests to plants that have not previously been sprayed.

To test how accurately prints made in this way represented the original spots as to size and shape, 100 measurements of the diameters of original spots were compared with measurements of the prints of the same spots. The spots after printing averaged  $5.6\mu \pm 2.1$  larger, indicating that the difference is small in any case and probably mostly due to errors in measurement. This was done with prepared paper that was just moist. With an excess of moisture present some enlargement or running of the spots occurs.

It would also be desirable to represent the relative coverage secured by different methods of spraying in some quantitative way. While one can make a fair estimate by comparing the appearance of prints made of leaves sprayed in different ways, such differences would be difficult to describe adequately without some way of measuring them. A few measurements have been made to test the practicability of the method. The spray spots on the prints were too small to measure conveniently directly. They were, therefore, enlarged. Outline drawings were made, using a camera lucida with a low power lens. To get the percentage of the total area covered by spray, the spots were cut out and weighed. A planimeter could be used for the same purpose.

Such measurements were made from a series of prints made in 1929 in spraying experiments. There were 3 series of plots: No. 1 sprayed at 200 pounds' pressure, No. 2 at 400 and No. 3 at 200 pounds, but with nozzles

TABLE 1.—*Showing the gain in yield and in percentage of leaf area covered by spraying at higher pressures and with larger nozzle openings*

Series compared	Pressures and gallons per acre	Average gain in percentage of leaf area covered	Average gain in yield, bushels per acre
No. 2 over No. 1	400 pounds (128 gals.) over 200 pounds (77 gals.)	$38.3 \pm 1.1$	36.5
No. 3 over No. 1	200 pounds (128 gals.) over 200 pounds (77 gals.)	$18.4 \pm 1.0$	18.1

of larger orifices, so as to apply as much material as at 400 pounds' pressure with the smaller-nozzle orifice. The results of this series of measurements are given in table 1, with differences in yields for comparison. The area figures in the table are the average differences for 10 measurements from the prints of the upper surfaces of the leaves at each pressure. This probably is an inadequate number, considering the variability likely to be encountered, but will at least indicate the possibilities of the method.

Thus it appears that the method devised of making prints from leaves, using paper prepared by moistening with an acidified potassium ferrocyanide solution, offers considerable promise. Such prints may be kept as a permanent record, and may serve as visual illustrations or may be used as a basis for more accurate study of the comparative covering secured with different apparatus or methods or on different parts of the plant.

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## PHYTOPATHOLOGICAL NOTES

*Physiologic Forms of Puccinia graminis tritici in Kwangtung, Southern China.*<sup>1</sup>—The black stem rust of wheat (*Puccinia graminis tritici* E. et H.) is the most destructive disease of wheat in Kwangtung Province, Southern China,<sup>2</sup> and is becoming the limiting factor in profitable wheat growing. From observations thus far made, there are no susceptible species of barley of any importance in this general region. Control by the eradication of the alternate host, therefore, is out of the question. Although dusting with sulphur has been demonstrated experimentally to control stem rust in other countries,<sup>3,4,5</sup> it is not feasible as a practical control measure in China. Apparently the only possible practical control measure will be by growing resistant varieties.<sup>6,7</sup> Consequently, a study of physiologic specialization of the pathogen is important.

During March and April, 1932, wheat infected with the uredial stage of rust was collected in various localities in Kwangtung Province and used for making inoculations on the 12 differential hosts listed by Stakman and Levine.<sup>8</sup> The usual methods were followed in inoculating, incubating, etc. The results are summarized in table 1.

It will be seen that there are 6 physiologic forms. Accession numbers 1 and 2 correspond with forms 15 and 9, respectively, as described by Stakman and Levine.<sup>8</sup> The remaining 4 have never before been described and appear, therefore, to be entirely new. In the latest key issued by Stak-

<sup>1</sup> The author is greatly indebted to Dr. E. C. Stakman and Dr. M. N. Levine, of the U. S. Department of Agriculture and the University of Minnesota, U. S. A., for furnishing seed of the differential wheat varieties and comparing the rust reactions with those recorded in their keys.

<sup>2</sup> Tu, Chih. Notes on diseases of economic plants in South China. Lingnan Sci. Jour. 11: 489-504. 1932.

<sup>3</sup> Bailey, D. L., and F. J. Greaney. Preliminary experiments on the control of leaf and stem rusts of wheat by sulphur dust. Sci. Agr. 6: 113-117. 1925.

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<sup>5</sup> Lambert, E. B., and E. C. Stakman. Effect of sulphur dust on the development of black stem rust of wheat in a natural epidemic. (Abst.) Phytopath. 16: 64-65. 1926.

<sup>6</sup> Aamodt, O. S. Breeding wheat for resistance to physiologic forms of stem rust. Jour. Amer. Soc. Agron. 19: 206-218. 1927.

<sup>7</sup> Hayes, H. K., and E. C. Stakman. Wheat stem rust from the standpoint of plant breeding. West. Can. Soc. Agron. Proc. 2: 22-35. 1922.

<sup>8</sup> Stakman, E. C., and M. N. Levine. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Sta. Tech. Bul. 8. 1922.

<sup>2</sup> Carter, Walter. The spotting of pineapple leaves caused by *Pseudococcus brevipes*, the pineapple mealy bug. *Phytopath.* 23: 243-259. 1933.

The absence of Smooth Cayenne is in itself significant, however, since the prevailing opinion among informed persons in Jamaica is that the variety disappeared on account of its extreme susceptibility to "wilt," the term being understood only in a generic sense. The variety Red Ripley, though highly regarded in Jamaica, is likewise much more rare than it was at one time. The variety most commonly grown is variously known as Cheese, or Sugar Pine. A number of other varieties are present in very small numbers in private gardens.

The conclusion was reached that mealy-bug wilt is present in Jamaica and that Red Ripley is becoming rare on that account. It is altogether likely that Smooth Cayenne has disappeared from cultivation there for the same reason. Cheese Pine is unquestionably much more resistant to mealy-bug wilt than any other variety seen. Even with high populations of mealy bugs this variety is vigorous and apparently wilt resistant.

Green spotting was encountered in Jamaica on several varieties. On those with pure green leaves the spots were typical of those described from Hawaii; on those whose leaves contained red and brown color, the color of the spots was modified. Data on green spotting are summarized in table 1.

TABLE 1.—Data on green spot of pineapples in Jamaica, September, 1932

Location	Variety	Green spots	Type of spot
Manor House	Cheese	-	Typical
	Porto Rican	-	Typical
	San Clarke	+	Flat; brown when old
	Cowboy	-	Spots show as darker brown areas on leaves that are deep green with brown underlay
Buff Bay	Cheese	-	
Orange Bay	Cheese	-	
Hope Gardens	Cheese	-	
	Ripley	-	
Half Way Tree	Cheese	-	Typical

In Guatemala, where collecting was done under a wide variety of environment, no green spotting was encountered, even though on the lowlands the pineapple mealy bug (*Pseudococcus brevipes* Ckl.) was very common and on a variety that in Jamaica showed green spotting, i.e., Cheese Pine. Since, in the areas where the mealy bug was common, the variety grown was almost exclusively Cheese Pine, the absence of mealy-bug wilt was not surprising in view of the Jamaican experience.

In Spanish Honduras, green spotting was found only in one place, The

United Fruit Company garden near Progresso. There the mealy bug was rare, but green spots were found on 3 varieties. It is perhaps significant that transfer of planting material from Hawaii to this area had taken place when The United Fruit Company made the variety collection. As a matter of fact, areas where green spotting has been observed have all shared in the transfer of planting material at one time or another. Since mealy bugs are readily transferred in this manner, the distribution is readily understood. A considerable amount of Smooth Cayenne material was transferred from Jamaica to Hawaii some years ago. There is some evidence that transfers had been made from Florida to Jamaica even earlier. Some interesting speculation as to the origin of mealy-bug wilt and green spotting is possible when its present distribution is considered—whether the two diseases have been transferred from one of these places to the others through the medium of mealy-bug-infested planting material, or whether, since the capacity to produce both appears to be inherent in the mealy bug, the greater severity and incidence of these diseases in Hawaii are associated merely with the higher state of pineapple cultivation existing there.

No typical wilt was observed in Spanish Honduras, though, again, the variety was principally Cheese Pine. In The United Fruit Company garden, mealy bugs were extremely rare, as was the case in the same company's experiment-station garden at Lancitilla, where a small pure stand of Smooth Cayenne was growing without any evidence of wilt whatsoever.—WALTER CARTER, Pineapple Experiment Station, University of Hawaii, Honolulu, Hawaii.

## BOOK REVIEWS

Paul Sorauer, *Handbuch der Pflanzenkrankheiten*. Herausgegeben von Dr. O. Appel. Volume I. Die Nichtparasitären und Virus-Krankheiten. Part I. (Nonparasitic diseases.) Pp. X + 592. Paul Parey, Berlin, 1933. 46.00 Gold Marks.

Sorauer published his manual on plant diseases a little more than half a century ago. All the facts were then crowded into a relatively small book, while the present edition consists of 6 formidable volumes, of which the first is issued in two parts of approximately 500 pages each. The subject matter of the first volume closely resembles Sorauer's first manual as it deals with non-parasitic and virus diseases. Through the most able editorship of Dr. Appel and his contributors there has been made available to the reader a wealth of information which makes the present edition an indispensable item in the library of the pathologist.

Part I of the first volume deals with nonparasitic diseases. It is divided into two sections. The first section is introductory and general in scope, dealing with the history of plant pathology, the concept of disease and the many-sided relationships between disease factors and environment. It is by far the most fascinating part of the first volume as it furnishes the physiological and ecological background for plant-disease study. It is written in a clear and forceful language, much easier to read than "Sorauer of old" whose long sentences often remained a mystery to those who boasted of only a high-school or college acquaintance with the German language. The second section of the book takes up: plant nutrition in relation to disease, climatic factors as causes of nonparasitic diseases and temperature extremes (heat and cold) as factors in death and disease.

The history of plant pathology is traced back to 4000 B. C. and the treatment of the subject is as complete as 79 pages of crowded print can make it. Its wealth of information far exceeds that found in related books and the story makes interesting reading not only to the pathologist but to all those interested in the progress of biological science.

The subject of general pathology, pp. 80–198 in the book, is intended as an introduction to the study of specific diseases. Following a brief, though clear, discussion on the concept of disease, approximately 20 pages are devoted to disease symptoms. The following 7 major types are listed and illustrated:

1. Wilting.
2. Discolorations (general and partial discoloration; spotting).

3. Death phenomena (dropping and withering of plant organs, rots, and decays).

4. Form changes (changes in size, simple form changes, atrophy, proliferation, etiolation, monstrosities, new formations).

5. Wounds (wounds due to atmospheric influences, gnarls and cancers, injuries resulting from animal feeding).

6. Deposits and excretions (honey dew; root pressure exudate, gum and resin flux, mucilage formation).

7. The presence of epiphytes and parasites as principal symptoms.

Two principal disease causes are recognized: a. inanimate nature acting either directly or indirectly, and b. living organisms, especially fungi. As principal inanimate causes the authors list unfavorable climatic and soil factors, presence of wounds and chemical influences.

The subject of parasitism and pathogenicity is treated in great detail. In the chapter on disease classification the different principal systems, especially those of Appel and Westerdijk and Wetzels are described and evaluated. Since a satisfactory explanation for the cause of disease is only obtained if the behavior of host and parasite to their environment is recognized and correctly evaluated, the part of the book dealing with ecological factors in relation to disease is most interesting. The authors give first a brief discussion on general plant ecology treating in some detail the subjects of: phenology, constitution and disposition, predisposition, degeneration and running out, immunity and resistance. In the chapter "on pathogen and environment" the subject of aggressiveness and virulence is taken up with special reference to the effect of temperature and nutrition. Attention is being called to the oligodynamic fertilizers such as manganese, boron, etc., which, in small quantities are stimulating, while larger applications are poisonous. Deficiency in boron may cause dry rot in beets, but, on the other hand, reduce *Helminthosporium* leaf spot of barley.

About 20 pages of the book are devoted to a discussion of the relation of animal parasites to environment; the mass appearance of insects and the subsidence of epidemics.

A diagnosis of nonparasitic diseases is difficult at best and often it is quite impossible if such material is sent to the laboratory away from its environment. To aid in the identification of such diseases pathologists have, in the course of years, accumulated a wealth of useful data and information which has been compiled and made available in the chapter on "plant nutrition and plant diseases." The authors treat first general symptoms, pointing out the fallacies in diagnosis from color symptoms. Under biochemical symptoms we become acquainted with pathological mineral metabolism; pathology of under-nutrition and other deficiencies, pathology of over-nutrition; pathological stimulation, pathological enzymatic processes and auto-

intoxication. Approximately 50 pages are devoted to mineral deficiency and the resulting effects on the plant. The need for a well-balanced soil solution has always been recognized, but it is only after reading this chapter in the book that one begins to see the numerous pitfalls the pathologist encounters in trying to diagnose a sick field or a sick individual growing on a deficient soil. A certain number of plants demand a finer ecological balance in relation to their environment than others. Notable examples are those that cannot grow on lime or acid soils.

With a knowledge of plant nutrition in relation to plant diseases for a background, the authors discuss in some detail a few of the economically important nonparasitic diseases: heart and dry rot of beets, dry-spot disease of oats, white head of oats and grasses, and reclamation (or moor) disease.

About one fourth of the book is given to a discussion of climate and weather as causes of nonparasitic diseases. Dr. Hiltner discusses first the subject of light and radiation, treating in some detail photoperiodism, time of seeding, light deficiencies, the effect of varying  $\text{CO}_2$  content of the air, and cosmic influences. Next he takes up temperature relations giving numerous examples of the effect of temperature excess or deficiency on the development of economic plants. Often the effect of temperature deficiencies is indirect. Thus, the flower abortion in grapes during a cold spring is not the direct effect of the cold but is due to a loss of balance in the absorption of minerals and photosynthesis. Of more than passing interest is also the chapter on water movement. Abnormally high humidity of the air is responsible for the formation of intumescences, abnormal cork proliferation, and warty protuberances, on stems. On the other hand, a very dry air may cause injuries to leaves and buds and is in part responsible for dry spot diseases, withertip and Weissährickeit (white heads in cereals). The last 100 pages of the book deal with temperature extremes as cause of death and disease in plants. The various theories on ice formation in plants are critically discussed and evaluated. What changes take place in the cell as the temperature drops, and is the plant able to endure such changes? As positive changes are listed: intercellular and intracellular ice formation, dehydration of cell colloids and changes in the salt concentration of the cell. Often the manner of defrosting exposed plants determines whether they will live or die. An explanation for this curious phenomenon is as yet lacking. Another unsolved problem is the freezing of plants above actual freezing temperature as frequently happens with tropical plants. Freezing often results in injury to both herbaceous and woody stems, causing splitting and malformations. Seeds may suffer decrease in germinability and frozen or injured organs often form favorable places for parasites to attack.

Injury and even death to plants and plant organs may be occasioned by temperatures above the normal. A common heat injury is the burning of

foliage often observed in greenhouses after a period of intense solar radiation. Leaf drop due to heat sometimes occurs in the spring and may be so severe as to produce a pathological aspect. The actual cause of this phenomenon is still obscure. Excessive cold and heat alike tend to weaken the plants and make them more easy prey to attack by parasites.

As may be seen from the above discussion the first part of Volume I deals to a large extent with normal plant physiology, an understanding of which is essential in the correct diagnosis of nonparasitic plant diseases. The literature citations are numerous, though mostly German. The illustrations are pertinent and mostly from the pen of Sorauer, thus vouching for their excellence.—ERNST ARTSCHWAGER.

*Recent Advances in Agricultural Plant Breeding.* By H. Hunter and H. Martin Leake. P. Blakiston's Son & Co., Inc., Philadelphia, 1933. 361 pp. \$4.00.

This volume, with a foreword by Sir Rowland Biffen of Cambridge, is written by men with long and fruitful experience in plant breeding and agricultural science. Hunter was in charge of plant breeding (barley) in Ireland for some 18 years and now has charge of oats breeding in the Plant Breeding Institute at Cambridge. Leake has intimate, first-hand knowledge of tropical crops, having served as Director of Agriculture, United Provinces, India, and as Principal of the Imperial College of Tropical Agriculture, Trinidad.

In his foreword Professor Biffen refers to the attempts now being made, by those engaged in economic plant breeding, to mitigate the losses caused by fungoid and insect pests. He states that "a considerable body of evidence has now been accumulated, that, even, if it is not often known what susceptibility and immunity to attack are actually due to, these factors are nevertheless heritable. Thus in section after section of this book it will be found that breeding for disease resistance is part and parcel of the investigator's work. This is notably the case with such crops as cotton and wheat. It is now clear that only continuous effort along clearly defined lines is required for obtaining a far greater measure of control over plant diseases than was ever dreamed of before Mendel's work set the course for the modern plant breeder."

The authors have adopted an interesting and significant basis for the selection of material and have "attempted to present in a connected form the results of only such investigations as have advanced beyond the limits of purely academic interest and the outcome of which has been the introduction of improved varieties into general agricultural use."

The 17 chapters of the book are grouped in two parts, (1) crops of the temperate regions, and (2) crops of the sub-tropical and tropical regions.



Many additional cases of resistance to plant diseases and insect pests of crops of the sub-tropical and tropical regions are described in Part II. Space permits mention of only a few examples. Thus, Java strains of cacao exhibit varying, but marked, degrees of resistance to disease, particularly to *Acrocercops cramerella*, the cacao moth, and to *Helopeltis*.

The stimulus that led to the very extensive work with sugar-cane seedlings arose from the disease susceptibility of the older canes. Resistance to the sereh disease in Java has been obtained by using the resistant *Saccharum spontaneum* as one parent in crosses. The Indian canes generally exhibit a marked degree of resistance to mosaic and, for this reason, Uba has become the predominant cane in South Africa. There are notable differences in the degree of resistance of citrus fruits to gummosis and withertip.

Studies of resistance to black root rot, and to black shank of tobacco, are described. In India Leake observed a considerable range of susceptibility to the leaf fungus, *Phytophthora*, in different races of the opium poppy. The races from Central India are notably susceptible.

With reference to maize, the authors state that "the question of disease is of importance second only to the question of inherent capacity to yield." Studies of resistance to corn smut, rust, pink rot, and Stewart's disease, are described. Roubaud reports complete immunity from the corn borer for Dent de Cheval and partial immunity for Hatif d' Auxonne maize, in France. Marston, of the Michigan experiment station, is using the borer-resistant maize Amargo from South America, to produce resistant varieties adapted to Michigan conditions. The studies of Collins and Kempton, of the United States Department of Agriculture, on infestation of corn earworm as related to the length and thickness of husk, are briefly described.

Varieties of rice show different degrees of resistance to blast, *Piricularia oryzae*, though none are immune. Studies of Reed and of Tisdale, *et al.*, on resistance to kernel smut in sorghum, are given brief mention.

The cotton plant is, in all countries, subject to numerous diseases that, in more than one instance, have become the critical factor in economic cultivation. Among the varieties resistant to wilt are Dillon, Dixie, and Dix-Affi. Strains of Pima and Acala cottons have been raised that are relatively resistant to cotton root rot. Harland has shown that Sea Island cotton responded to selection for resistance to *Bacterium malvacearum* E.F.Sm. Distinct progress has been made by cotton breeders in producing varieties resistant to insects. Thus, Harland has reported distinct differences in palatability of different species and varieties of cotton to the boll worms. Early maturing strains of long-staple cotton such as Express and Webber have helped make it possible to grow Upland varieties in the United States under boll-weevil conditions. In the West Indies, the Upland

In the first 8 chapters, plant-breeding results with wheat, barley, oats, flax, the potato, forage grasses, red clover, and white clover, and with root crops—mangel, swedes and turnips—are described. Chapters 9 to 17 deal with beverages—coffee, cacao, tea; sugar cane, fruits, including citrus and allied genera and the banana; tobacco, the opium poppy, the tropical cereals: maize, rice, and sorghum; rubber, fibres: cotton, sisal, New Zealand flax, jute; and oil plants: coconut, oil palm, castor, groundnut, sesame, and the soy-bean. It may seem a bit strange to plant pathologists and plant breeders in the northern United States to see maize classed as a tropical cereal, but this is perhaps not illogical from the standpoint of workers in the British Empire, in whose hands this book will probably find its greatest usefulness. The literature of plant breeding with tropical crops has heretofore been widely scattered and not readily available, and the authors have rendered a real service in bringing together much of this very interesting material.

Throughout the book emphasis is given to the problems, methods, and results in breeding crop plants for resistance to plant diseases and insect pests. Plant pathologists, plant breeders, entomologists, and agronomists, who are interested in this field will find the book a very useful and convenient reference work.

In the chapters on wheat, considerable space is devoted to discussion of the early studies of Biffen on rust resistance and to more recent work of Stakman, Hayes, Waldron, Clark, McFadden, Goulden, *et al.* Reference is made also to the studies of Painter and his associates at the Kansas station, on the resistance of varieties of winter wheat to Hessian fly.

The work of Danish, Irish, and English investigators is featured in the chapter on barley, which includes a succinct discussion of malting quality.

Studies of resistance to the smuts, rusts, grey-spot disease, and to the fruit fly, *Oscinella Frit.* Linn., are described in the chapter on oats, where the publications of Reed, Gaines, Stanton, Murphy, and other American workers, and of European investigators at Cambridge, Oxford, Aberystwyth, and Svalof are cited.

Studies of resistance to wilt and rust are described in the chapter on flax, in which some of the papers of Bolley, Stakman, and other American workers, and of Pethybridge in Ireland, are reviewed.

Studies of blight resistance by Müller, Reddick, *et al.*, and of wart disease by Salaman and Lesley, and virus diseases by K. M. Smith, are described in the chapter on the potato.

A strain of rye grass known as Hawkes Bay is described that shows marked resistance to leaf rust, *Puccinia coronata*. Davies *et al.* in Wales have described varieties of swedes resistant to the finger and toe disease, *Plasmidiophora brassicae*.

group of cottons are all severely attacked by the blister mite. The Bourbon group is immune, while the Peruvian group shows varying degrees of susceptibility. In South Africa the attacks of the cotton jassid are so severe that it seemed probable that cotton cultivation throughout the southern portion of that continent was doomed. Worrall and Parnell have selected resistant strains. One of these, U4, is markedly superior to all the others and in 1929-30 practically the whole of the low veldt was planted with it. Cambodia cotton, a type introduced into South Africa from India, has proved even more resistant to jassid than U4. A close correlation has been noted between resistance to the jassid and hairiness of the cotton plant. Certain strains of Sea Island cotton show a marked degree of resistance to leaf curl, a virosis.

There are noteworthy differences in resistance to yellow leaf, a virosis of New Zealand flax.

If any plant pathologist or entomologist doubts the importance of plant breeding as a control measure and if any plant breeder is unaware of the accomplishments and opportunities in breeding for resistance to diseases and insect pests, let him turn to "Recent Advances in Agricultural Plant Breeding" by Hunter and Leake, for inspiration and enlightenment.—JOHN H. PARKER, State College of Agriculture, Manhattan, Kansas.

*Environmental Factors in Relation to Plant Disease and Injury: A Bibliography.* By J. D. Wilson. Ohio Agr. Exp. Sta. Tech. Bul. 9. 203 pp. 1932. Price, \$1.00.

In the progress of any branch of science a periodic inventory of the details amassed by many workers in diverse fields is essential in order that the facts may be consolidated and the true objectives more clearly perceived. Biologists generally have adhered far less closely to a *grand plan* of research than workers in the more exact sciences—physicists for example in their definite quest to understand the organization of matter. We say that the great aim of biology is "to explain life," but in plant pathology at any rate, we continue to regard as our serious business the descriptive and therapeutic phases of plant diseases. Perhaps this is because plant pathology is superficially only one of the phases of crop production and utilization; possibly another factor is the, as yet slight, progress of its synthetic development. To the extent that comprehensive inventories of accumulated data are prerequisite for the synthesis of principles, noteworthy aid to such synthesis in plant pathology is afforded by the publication of an extensive bibliography on the relation of environmental factors to plant disease and injury.

The subject of environmental relations to plant disease might appear as fairly extensive to even a well-informed layman. To a professional plant

pathologist it might well seem as too vast for any one to attempt except a student in Plant Path. I. For all the reviewer knows, the Wilson bibliography may be the outgrowth of one of these dreams such as ambitious beginners have. However, its present magnitude is convincing evidence that the author did not as predoctorate students often do, begin to "specialize" (*i.e.*, curb his ambition) as soon as the bibliography attained the burdensome size of several hundred entries. It requires rampant ambition to carry through to completion a bibliography of over 3,600 titles, as Wilson's does, and it takes an exceptional degree of organizing skill to provide effective clues to so great a body of data.

The 3,689 literature citations are arranged alphabetically by authors, and are numbered serially. Next follows an "environmental factor index," with the major factors listed under 24 headings including the usual meteorological and nutritional factors, and also categories not ordinarily included in the environmental complex—host characters, various deleterious agents such as toxins, smoke, dust, injuries from control operations, and the time factor in viability of pathogens. The major factors are subdivided into more specific categories, which are repeated in a second list with the serial numbers of the literature citations arranged under each topic. For example the major factor "Hydrogen-ion relations" has the following subdivisions: A. General; B. Regulating disease. 1. Acid soil favoring, 2. Alkaline soil favoring, 3. Cardinal soil reactions for disease development; C. Regulating growth. 1. Cardinal reactions of medium for fungi, 2. Cardinal reaction of medium for other plants, 3. Plant growth and distribution. The group C-1 contains 158 numbers each referring to a title in the literature under which cardinal reactions of media for growth of fungi are discussed. The relations of temperature to spore germination include some 200 citations, to growth nearly 500, lethal effects 200, etc.

A second index lists host plants alphabetically (by common and Latin names) and under each the diseases (descriptive name and Latin name of pathogen) and other injuries reported in literature. Under each disease or injury, the serial numbers of pertinent literature citations are given. Aside from the clues to literature that it furnishes, this index is a useful compilation of host plants and their diseases. As this index covers some 27 pages, a final host index listing the page on which each host appears is provided.

Noteworthy complete and skillfully organized, this bibliography will prove an indispensable reference work to student and professional plant pathologists.—FREEMAN WEISS, United States Department of Agriculture, Washington, D. C.

## FIRST INTERNATIONAL CONGRESS OF ELECTRO- RADIO-BIOLOGY

For the purpose of instituting among physicists, chemists, biologists, naturalists and doctors a close and profitable collaboration, indispensable for the advance of radio-biology considered not as a branch of radiology or of biology, but as a separate science in itself, the International Society of Radio-Biology is now preparing the organization of the First International Congress of Electro-Radio-Biology, which is to take place in Venice, in the Ducal Palace, in September, 1934, under the presidency of H. E. Count Volpi di Misurata, Minister of State.

All the subjects concerning oscillatory and corpuscular phenomena in relation to biology will be examined and discussed: ultra-sounds, electric waves, infra-red, light, ultra-violet, radium, penetrating radiation in its probable influences on the various manifestations of organic and organized matter and in applied laboratory work; photodynamic actions, long-distance actions of metals, Gurwitsch rays, phenomena of luminescence, radiations of radio-active salts in organic combinations; electric states of the atmosphere; spectrographie; influences of the radiating energy on heredity, etc. These are the subjects of the greatest importance, which will constitute a vast experimental and theoretic whole, to be treated by specialists in physics, in physical chemistry, in radiology, in biology, in botany, in zoology, in medicine, etc.

It is therefore a fundamental congress based on new and universal foundations, in which, for the first time, those whose studies are directed on all matters having a direct or indirect relation with the subject will exchange their own ideas, intending thus to reach a program of unification in the promising field of radio-biology.

The most prominent figures in physics, chemistry, and present day biology will take part, and they will give lectures on physics, chemistry and biology. After these lectures will follow talks of a more strictly radio-biological nature. Every radio-biological talk will be delivered separately by specialized physicists and biologists, through which the congress may, finally (after due consideration of the talks, discussions, and communications of the sections), be able to reach a radio-biological synthesis founded on severe principles of physics, physical chemistry, and biology.

The following will be present at the Congress and will give lectures and speeches: Emil Abderhalden (Halle A. S.); Belak (Budapest); Brunetti Rita (Cagliari); Arthur Compton (Chicago); Coolidge (Schenectady, N. Y.); Ducceschi (Padova); Glasser, Otto (Cleveland, Ohio); Gola

(Padova); Gurwitsch (Leningrad); Haskins (Schenectady, N. Y.); Magrou (Paris); Marinesco (Bucharest); Nadson (Leningrad); Nemenow (Leningrad); Palmieri (Bologna); Perussia (Milano); Pincussen (Berlin); Pugno-Vanoni (Padova); Rahn (Ithaca, N. Y.); Raman (Bangalore-India); Reche (Leipzig); Roffo (Buenos Aires); Rossi, Bruno (Padova); Swend Lomholt (Kopenhagen); Stempell (Münster); R. W. Wood (Baltimore), and other names will be added in successive communications.

In order to coordinate the tendencies of the congress-members in the best way possible, and at the same time to make present to the National Research Council the founding and the development of the initiative, those who desire more detailed information are invited to apply to the temporary head-office of the International Society of Radio-Biology, addressing their correspondence to: Dr. Giocondo Protti, Venice (Italy), Canal Grande-S. Gregorio 173.

# PHYTOPATHOLOGY

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## STUDIES ON ACQUIRED IMMUNITY WITH TOBACCO AND AUCUBA MOSAICS

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Most plants that became infected with virus diseases retain the etiological agents as long as they live. If they acquire immunity, it must be of the nonsterile type, such as is known for certain viroses of animals that do not show the usual sterile type of immunity.

Because of their wide host ranges and the ease with which they may be transmitted, plant virus diseases are well suited for studies on immunity. This is especially true of the systemic ones that do not kill plants, for they can be held under observation for long periods of time. Moreover, there is evidence that such diseases may be limited in their severity by some mechanism which prevents undue multiplication of the infective principle.

The occurrence in nature of closely related strains of certain virus diseases would seem to offer favorable possibilities for cross-immunization studies, since the strains frequently show considerable differences in severity. It would be desirable from both the practical and scientific viewpoints if it could be shown that plants infected with one strain are thereby protected against infection by other strains, including those of greater severity. Studies were, therefore, undertaken for the purpose of determining whether plants inoculated simultaneously with two viruses that are presumed to be related strains of tobacco mosaic will come down with both or with only one of the strains, and whether plants having either of the two strains can be infected with the other. The ordinary field type of tobacco mosaic and aucuba mosaic of tomato were used in the experiments to be reported in this paper.

### LITERATURE

*On Acquired Immunity in Plants.* A review of the literature on acquired immunity in plants has recently been published by Chester (9). A few of the most important papers on acquired immunity from viroses of plants will be mentioned here.

Thung (28) isolated a yellow mosaic believed to be similar to McKinney's (16) yellow mosaic of tobacco and Johnson's (13) tobacco virus

<sup>1</sup> Published at the expense of The Rockefeller Institute for Medical Research out of the order determined by the date of acceptance of the manuscript.

No. 6. When approximately equal parts of juice containing the yellow-mosaic virus and juice containing the common tobacco-mosaic virus were mixed and the mixtures used to inoculate healthy tobacco plants, the symptoms of both diseases appeared in the inoculated plants. From certain spots in the leaves the infective principle of each disease was separately recoverable. If a light yellow spot was cut out of a leaf and used in the inoculation of healthy plants, the yellow mosaic was always recovered. If a bit of tissue from a darker green area was used, the ordinary green mosaic was isolated. This led Thung to conclude that only one virus could occupy any given cell. When a plant having yellow mosaic was inoculated with tobacco-mosaic virus, it failed to develop the symptoms of this disease. Likewise, when a plant having tobacco mosaic was inoculated with yellow-mosaic virus, the symptoms of the latter failed to appear. A single exception is mentioned. One plant having tobacco mosaic at the time it was inoculated with yellow-mosaic virus developed the symptoms of both diseases. Thung's work indicates that either, but only one, of the two viruses can multiply extensively in any given cell. His only means of testing for the presence or absence of either virus was to subinoculate to healthy tobacco plants and await the appearance of symptoms. He could easily prove by this method that the two viruses were not present in equal proportions in any selected group of cells. But he had no way of showing whether or not small amounts of either virus might be present in cells containing large amounts of the other.

Price (17) found that plants of the four species, *Nicotiana langsdorffii* Schrank, *N. sylvestris* Speng., and Comes, *N. quadrivalvis* Pursh., and *N. tabacum* L., regularly recover and are subsequently immune from the ring spot of tobacco. Although most recovered plants could not be distinguished from those that had not suffered an attack, they were invariably found to harbor the virus of ring spot. Juice from such plants proved to be highly infectious. Price showed that immunity from ring spot persists through at least three generations grown from cuttings but is not transmitted through seeds. The symptoms of tobacco mosaic in recovered plants were observed to be less severe than in plants free of the ring-spot virus.

Salaman (18) has recently reported the isolation of two strains of the X virus of potato. One strain, designated as the L type, causes a severe disease with intense mottling and dwarfing. The other, described as the G type, gives an almost imperceptible reaction. In both tobacco and *Datura stramonium*, the mild G type virus protects plants against infection by the severe L type virus. It does not, however, protect them against infection by the Y virus of potato or by the virus of ordinary tobacco mosaic. Here again is evidence that two closely related strains of a virus can not occupy the same plant tissue, although they are not antagonistic to unrelated viruses.



Botjes (4) has shown that when plants of certain potato varieties are infected with either secondary topnecrosis or attenuated secondary topnecrosis, they are, as a rule, protected against primary topnecrosis even though inoculated with the virus of this disease by means of grafting. The former produce mild symptoms and are apparently attenuated strains of the latter. Botjes' work brings evidence that potato plants infected by attenuated strains of topnecrosis are not invaded by unattenuated virus except in a small percentage of cases. Botjes states that the virus of topnecrosis is known in America as the healthy-potato virus. Since Kenneth Smith (27) believes his X virus to be one of the so-called healthy potato viruses first described by Johnson (11), it is possible that Salaman and Botjes worked with strains of the same disease.

East (10) has suggested that recovery of sugar-cane plants from mosaic may be due to acquired immunity, and has reported experiments believed to support this view.

Insofar as the writer is aware, the best evidence at present available in support of a belief that plants may acquire immunity from a virosis by virtue of having suffered an attack of that or of some closely related virosis, is found in the papers cited above. Thung, Salaman, and Botjes obtained evidence of acquired immunity in plants by making use of related strains of virus diseases in cross-immunization tests. Price obtained similar evidence by using a disease from which plants regularly recover.

Silberschmidt (24) made extensive studies on the problem of resistance to and immunity from tobacco mosaic. He was unable to obtain evidence of antibodies or to demonstrate either active or passive immunity in the plants studied.

Birkeland (3) reports experiments indicating that *Nicotiana glutinosa* L. does not develop an acquired immunity from infection by either the virus of tobacco mosaic or that of spot necrosis. Plants infected with tobacco mosaic and tested with spot necrosis, as well as plants infected with spot necrosis and tested with tobacco mosaic, gave similar results.

*On Aucuba Mosaic of Tomato.* Aucuba mosaic of tomato was first reported by Bewley (2) in 1924. He noted that it causes more intense yellowing of foliage than does ordinary tomato mosaic. He also observed that it differs from the latter in causing tomato fruits to mottle. The disease has been studied by Smith (25, 26), Sheffield (20, 21, 22, 23), and Caldwell (5, 6, 7, 8). Smith (25) has pointed out that, in its general characteristics and in the nature of the disease it produces, the virus of aucuba mosaic corresponds very closely to that of tobacco mosaic. He states that aucuba mosaic differs from the latter disease in the much greater intensity and brilliance of leaf symptoms but emphasizes the fact that it is similar to tobacco mosaic in filterability and in resistance to heat, alcohol,

dilution, and aging. He also notes a similarity in its ready transmissibility by inoculation with juice, as well as by insects. Later studies on filtration indicate, however, that the virus particle of aucuba mosaic is larger than that of tobacco mosaic (15).

The writer (14) has reported that aucuba mosaic differs from ordinary tobacco mosaic in its capacity to produce chlorotic primary lesions in the leaves of many varieties of tomato and chlorotic or necrotic primary lesions in the leaves of certain species and varieties of *Nicotiana*.

#### MATERIALS AND METHODS

The method of inoculation used in the experiments to be reported was the same in all cases, except where mention is made of a different method. Small glass spatulas similar to those described by Samuel (19) were used in applying inoculum. The flattened ends of the spatulas were covered by a layer of cheesecloth to prevent too much wounding of the leaf surfaces inoculated. The cloth was stretched tightly and smoothly over the ends of the spatulas and tied on with thread. All spatulas were covered with new cloth and sterilized in an autoclave before being used. Each inoculum was prepared by grinding small pieces of leaf tissue in a sterile mortar. Juice thus obtained was used to wet the cloth-covered ends of the spatulas. Except where otherwise stated, plants were inoculated by gently rubbing fresh juice from diseased plants of *Nicotiana tabacum* over the entire upper surface of a mature young leaf. After each inoculation excess inoculum was removed by washing the rubbed parts in a spray of water.

The plants were grown in soil in clay pots immersed in moist peat on benches in a greenhouse. The greenhouse was fumigated at frequent intervals to control insects. Before inoculation all plants were moved from the house in which they had been grown to an experimental greenhouse. Most of the experiments were made in the summer and autumn when the greenhouses were not heated, except for a few days in September and October. The temperature in the houses varied over a wide range, but is believed to have gone no lower than 65° F.

The plants used were in good growing condition. They were all of the species *Nicotiana glauca*, except where special mention is made of other species. The tobacco-mosaic virus was that designated by Johnson (13) as tobacco virus No. 1. It was obtained from James Johnson several years ago. The virus had been passed through tobacco plants many times before being used in the experiments here reported. The aucuba-mosaic virus was obtained from Cornell University through the kindness of H. H. Whetzel who got it from the Rothamsted Experimental Station, Harpenden, England. It also had been passed through tobacco plants many times before being used in the tests reported here.

The experiments to be described below have all been repeated one or more times. The results obtained in the repetitions agree closely with those reported.

THE SYMPTOMS OF TOBACCO AND AUCUBA MOSAICS ON  
NICOTIANA SYLVESTRIS

The mottling caused by tobacco and aucuba mosaics on most varieties of tobacco and tomato are much alike, except that aucuba mosaic produces leaf symptoms of greater brilliance and severity than those produced by tobacco mosaic. On mature leaves of *Nicotiana glutinosa*, *N. langsdorffii*, *N. rustica* L., *N. alata* Link and Otto, *N. sanderae* Sander, and the bean (*Phaseolus vulgaris* L.) varieties Refugee Green Pod, Robust, Scotia, Early Golden Cluster, Hodson Long Pod, and Ideal Market, the viruses of tobacco and aucuba mosaics produce necrotic primary lesions that are indistinguishable. On similar leaves of *N. sylvestris* and *N. tabacum*, varieties Adcock, Burley, and Connecticut Seed Leaf the virus of aucuba mosaic produces necrotic primary lesions, while that of tobacco mosaic causes faintly chlorotic primary lesions. Since systemic infection is associated with chlorotic primary lesions and does not ordinarily follow the production of necrotic primary lesions, the two diseases differ very sharply in their effects on these hosts.

The symptoms of tobacco mosaic on tobacco and a number of other host plants are recorded in a voluminous literature. Smith (25) has published an excellent description of aucuba mosaic on the tomato variety Kondine Red. The symptoms reported are similar to those shown by most other plants in which the disease becomes systemic. Any further general description is unnecessary. Since, however, the results of the experiments to be reported depend on the reaction of *Nicotiana sylvestris* to the viruses of tobacco and aucuba mosaics, a brief description of the symptoms of each disease as it appears on this host will be given.

Approximately four days after inoculation with tobacco-mosaic virus, primary lesions consisting of faint yellow spots appear on the leaf into which virus was rubbed. The spots vary in size from 1 to 2 mm. when first discernible. They enlarge gradually from day to day and, if numerous, may cause yellowing of the entire leaf. The intensity of yellowing in the primary lesions increases with the age of the lesions and the age of the leaf. The first symptom of systemic infection with tobacco mosaic is a clearing-of-veins in one or more leaves near the top of the plant. This is soon followed by mottling in young leaves. The old leaves are not mottled by the disease. The systemic symptoms are similar to those that have been described frequently for tobacco mosaic on tobacco.

If a young plant of *Nicotiana sylvestris* about 50 days' old is infected with the virus of aucuba mosaic by means of leaf inoculations, a result very

different from that following infection by tobacco mosaic is obtained. Necrotic primary lesions appear on the inoculated leaf in from one to two days, but all other leaves remain healthy in appearance. The necrotic lesions enlarge slowly but continuously until the leaf dies because of the enlarging lesions or of old age. If lesions are numerous, the leaf may collapse within 3 to 5 days after inoculation. The disease does not become systemic. All parts of the plant remain free of virus except the inoculated leaf. The differences in the symptoms produced by the two viruses are very conspicuous in *N. sylvestris*. Advantage may be taken of this fact to determine whether any sample of juice contains either or both of the viruses under study. The difference in the reaction of *N. sylvestris* and a few other plants to these two closely related viruses is unusual. A somewhat similar case has, however, been reported by Ainsworth (1).

#### ATTENUATED STRAINS OF AUCUBA MOSAIC

Aucuba mosaic has been maintained on tobacco and tomato for more than 2 years without change in symptoms. During this period the disease has been transmitted many times to *Nicotiana sylvestris*. When inoculated into the leaves of this plant, aucuba mosaic invariably has produced the necrotic primary lesions described above. It does not produce a general infection except in very young plants where it sometimes causes systemic necrosis. It never causes mottling. The conclusion that the disease does not vary and is due to a stable type of virus that may be maintained indefinitely is difficult to avoid.

It must be noted, however, that aucuba mosaic will become systemic if inoculated plants are held at a sufficiently high temperature for several days. Neither the minimum temperature nor the minimum time necessary to bring this about have yet been accurately determined. Young plants held at a temperature of about 35° C. for 3 days following infection with aucuba mosaic, invariably come down with a systemic form of the disease. When such plants are removed to greenhouses held at a temperature too low to initiate systemic infection, the systemic form of the disease persists. Three very distinct types or strains of aucuba mosaic have been obtained from plants incubated at high temperatures for various periods of time after inoculation.

The first strain closely resembles aucuba mosaic when transmitted to tobacco and tomato plants. But when inoculated into plants of *Nicotiana sylvestris*, it causes intense mottling with brilliant leaf symptoms such as are shown in figure 1, A. It does not produce necrotic primary lesions. It has been passed through *N. sylvestris* plants in serial transfers without apparent change. It has acquired the capacity to cause systemic infection, but has at the same time lost the ability to produce necrotic primary lesions.

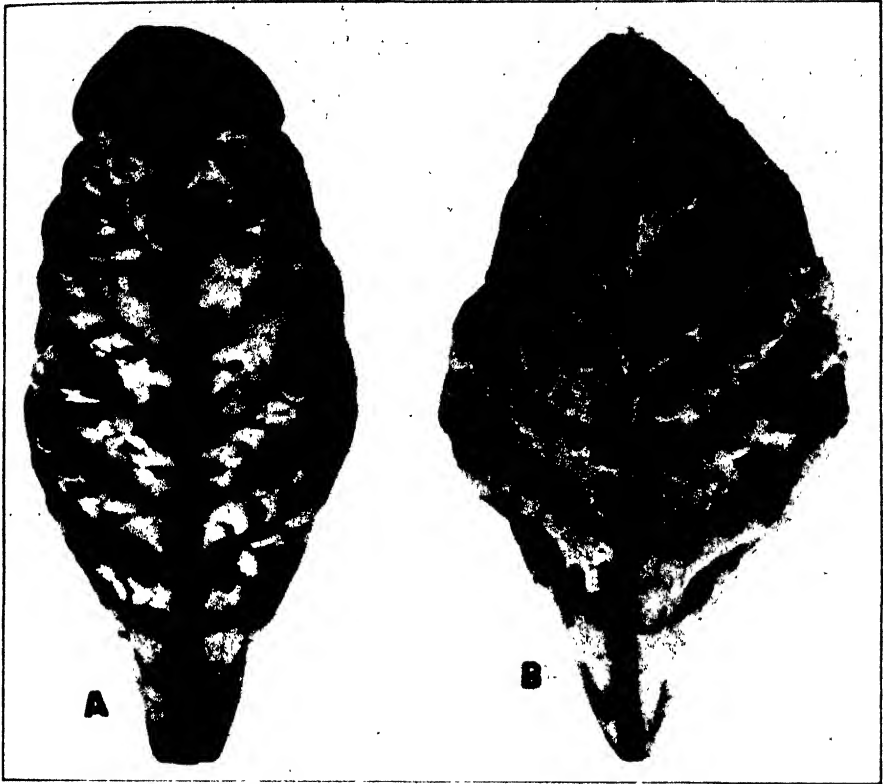


FIG. 1. A. Young leaf of *N. sylvestris* showing brilliant mottling due to an attenuated strain of aucuba mosaic. B. Leaf from a *N. sylvestris* plant having cucumber mosaic 5 days after it was inoculated with aucuba mosaic. Necrotic lesions have developed in both the light and dark green areas of the leaf. Cucumber mosaic does not give protection against aucuba mosaic.

The second strain differs from aucuba mosaic in that it does not produce on tobacco or tomato the brilliant leaf symptoms so characteristic of this disease. In fact, it is difficult to distinguish from tobacco mosaic on these plants. But when inoculated into *Nicotiana sylvestris*, it produces necrotic primary lesions typical of aucuba mosaic, and does not cause systemic infection.

The third strain differs very little from tobacco mosaic. On tomato and tobacco plants it causes a green mottling that is difficult to distinguish from tobacco mosaic. On *Nicotiana sylvestris* it produces no necrotic primary lesions, but causes mottling of the green mosaic type.

A number of other forms of aucuba mosaic have been obtained that may be characterized as intermediate strains. They will not be described here. All strains, including the 3 above described, are less severe than aucuba

mosaic on both tobacco and tomato plants. It is believed that the strains were derived from aucuba mosaic through attenuation by heat. They are, therefore, referred to as attenuated strains.

LEAVES MOTTLED BY ATTENUATED STRAINS OF AUCUBA MOSAIC  
IMMUNE FROM INFECTION BY UNATTENUATED VIRUS

Many tests have shown that mature mottled leaves of *Nicotiana sylvestris* plants, having either of the systemic attenuated forms of aucuba mosaic described above, are immune from unattenuated virus. When such leaves are rubbed with juice containing the virus of aucuba mosaic, no lesions whatever are produced, although a like inoculation of similar leaves of healthy plants invariably results in the production of numerous conspicuous necrotic lesions. The following experiment will serve to illustrate the manner in which the tests were made.

A mature mottled leaf on each of 10 plants infected with an attenuated strain of aucuba mosaic was rubbed with juice containing unattenuated virus. A similar leaf on each of 10 healthy plants was rubbed with another sample of the same inoculum. When the leaves were observed 4 days after their inoculation, it was found that numerous necrotic lesions had developed on all inoculated leaves of the previously healthy plants, but no lesions had developed on any of the mottled leaves. The experiment proves that leaf tissues mottled by attenuated virus become immune from infection by unattenuated virus.

MATURE LEAVES MOTTLED BY TOBACCO MOSAIC IMMUNE  
FROM AUCUBA MOSAIC

An experiment was undertaken for the purpose of determining whether the leaves of plants having tobacco mosaic might also acquire immunity from aucuba mosaic.

Each of 10 plants of *Nicotiana sylvestris* that had been infected with tobacco mosaic 57 days previous to the date on which they were selected for use in this experiment was inoculated by rubbing the virus of aucuba mosaic over the upper surface of each of 5 mature leaves. Each of 10 healthy plants of the same kind and age was inoculated in the same way. Ten similar plants left uninoculated served as checks. At the time of inoculation, the leaves of the plants having tobacco mosaic were typically mottled, slightly malformed, and somewhat stunted. In other respects they were similar to the leaves of the healthy plants. Both sets of leaves were approximately the same age.

Four days after their inoculation with the virus of aucuba mosaic the 50 inoculated leaves on the 10 previously healthy plants showed numerous necrotic lesions, such as are typical of the primary lesions produced by

this disease. The 50 mottled leaves on the 10 plants having tobacco mosaic were not visibly affected. No lesions of any kind developed on these leaves during the period of 6 weeks that they were held under observation. The uninoculated check plants remained healthy. Figure 2 shows a leaf from each set of the inoculated plants as it appeared 5 days after inoculation. The test proves that mature leaves of *Nicotiana sylvestris* plants having tobacco mosaic are immune from aucuba mosaic under the conditions of the experiment.



FIG. 2. Two leaves of *N. sylvestris* as they appeared 5 days after both were inoculated with aucuba mosaic. The leaf on the left was healthy, while that on the right was mottled by tobacco mosaic at the time of inoculation with aucuba mosaic.

In order to determine whether the virus of aucuba mosaic might have infected and multiplied in the mottled leaves without producing lesions, subinoculations were made from each of 2 of the inoculated leaves on each

of the 20 plants 7 days after their inoculation with aucuba mosaic. The subinoculations were made from discs of leaf tissue about 1 cm. in diameter, cut out of each of the leaves by means of a sterile cork border. Each disc was mashed in a few drops of water on a glass slide. The inoculum thus obtained was used to inoculate 2 leaves on each of 40 healthy young plants of *Nicotiana sylvestris*. Subinoculations from the 20 mottled leaves produced only 4 necrotic lesions. One lesion developed from inoculations with juice obtained from each of 4 discs. These lesions may have resulted from virus left on the surface of the discs when the leaves were inoculated 7 days earlier. The subinoculations from the 20 leaves bearing lesions produced necrotic lesions in every instance. A total of 127 lesions resulted from these inoculations. The subinoculations prove that the virus of aucuba mosaic multiplies little or none in the mottled leaves.

YOUNG LEAVES OF PLANTS HAVING TOBACCO MOSAIC NOT  
IMMUNE FROM AUCUBA MOSAIC

An experiment was made for the purpose of determining whether very young leaves of plants having tobacco mosaic are immune from aucuba mosaic.

Ten healthy young plants were inoculated with tobacco-mosaic virus. Ten similar plants left uninoculated served as checks. All of the 10 inoculated plants came down with tobacco mosaic. The 10 check plants remained healthy. Two weeks after the first appearance of symptoms in the tips of the plants, a young mottled leaf, about 1½ in. long, on each of the 10 plants was inoculated by rubbing the upper surface with juice containing the virus of aucuba mosaic. Similar leaves on the 10 check plants were likewise inoculated. One week after these inoculations, several necrotic lesions were observed on each of the mottled leaves. Most, but not all, of the lesions were on veins. Typical aucuba-mosaic lesions appeared on the inoculated leaves of all the check plants. They were, however, more numerous on these leaves than on the mottled leaves. The lesions on both the mottled and the check leaves enlarged slowly. Three weeks after their appearance, subinoculations were made from 10 of the necrotic lesions on mottled leaves to leaves of healthy young *Nicotiana sylvestris* plants. All of the subinoculations resulted in the production of necrotic spots typical of the primary lesions of aucuba mosaic. A total of 78 lesions resulted. The test brings evidence that the virus of aucuba mosaic multiplies in lesions on mottled leaves and proves that very young leaves of plants having tobacco mosaic are not immune from aucuba mosaic. The young leaves differ distinctly from mature leaves in this respect.



IMMUNIZATION BY DIRECT LOCAL INOCULATION OF  
MATURE LEAVES

On some of the plants used in the experiments described above, it was observed that leaves inoculated with tobacco-mosaic virus previous to inoculation with the virus of aucuba mosaic, bore fewer necrotic lesions than the other mature green leaves on the same plants. This suggested the possibility of immunizing mature leaves by direct inoculation. Experiments were, therefore, undertaken to determine whether the direct inoculation of full-grown leaves with the virus of tobacco mosaic would render them immune from aucuba mosaic.

A mature leaf on each of 10 healthy plants was inoculated by rubbing

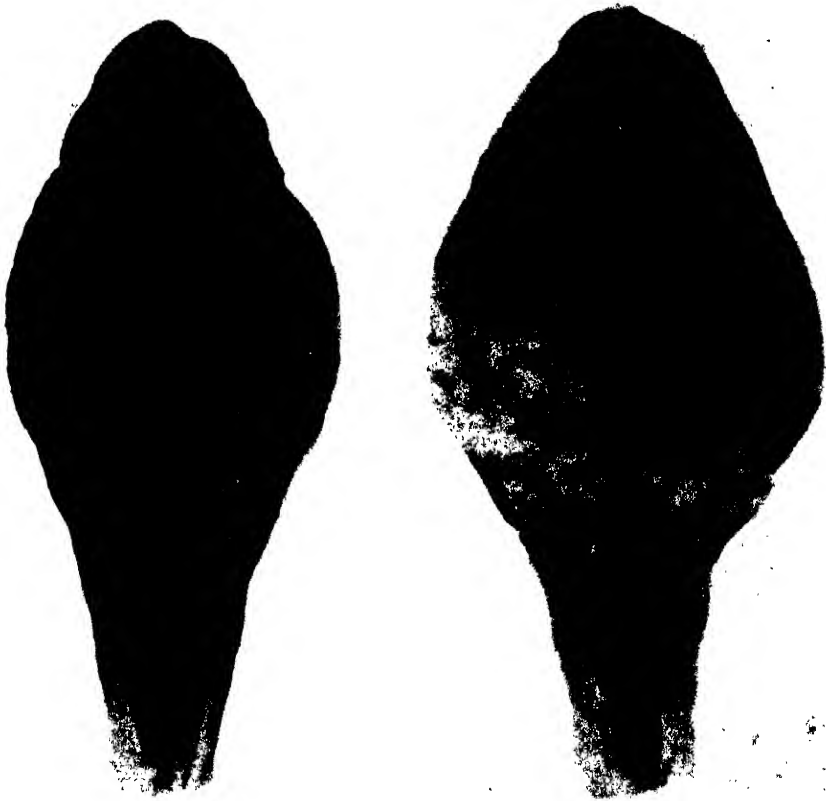


FIG. 3. Two leaves of *N. sylvestris*. The leaf on the right was rubbed with juice containing the virus of tobacco mosaic 5 days before both were inoculated equally with aucuba mosaic. The leaf on the right acquired immunity from aucuba mosaic as is shown by the absence of primary lesions in most of the tissues.

juice containing the virus of tobacco mosaic over the entire upper surface. Another mature leaf on each plant was rubbed with juice from a healthy plant. This was done in order to cause approximately the same degree of wounding on both sets of leaves. Five days later, all of the leaves were reinoculated by rubbing juice containing the virus of aucuba mosaic over their entire upper surfaces. When the leaves were examined 4 days after this inoculation, it was found that many necrotic spots typical of the primary lesions of aucuba mosaic had developed on the leaves rubbed with virus-free juice before they were inoculated, whereas very few necrotic spots had developed on the leaves inoculated with the virus of tobacco mosaic previous to inoculation with that of aucuba mosaic. It was observed that most of the necrotic lesions occurring on the leaves inoculated with both viruses were confined to areas that might have been missed in the first inoculation. Most of them were on the bases of the leaves where the second inoculation may have extended somewhat lower than the first. A set of leaves from one of the plants is shown in figure 3. The leaf on the left was rubbed with juice from a healthy plant, that on the right with juice from a plant having tobacco mosaic, 5 days before both leaves were inoculated equally with aucuba mosaic. It will be seen that necrotic lesions are well distributed over the leaf rubbed with healthy juice, but are confined chiefly to the base of the leaf rubbed with juice containing tobacco-mosaic virus. The experiment proves that mature leaves inoculated with tobacco-mosaic virus acquire immunity from aucuba mosaic.

#### IMMUNITY CLOSELY ASSOCIATED WITH INVASION BY THE VIRUS OF TOBACCO MOSAIC

In the description of tobacco mosaic on *Nicotiana glauca*, it was pointed out that mottling occurs only in those leaves that are very young at the time of infection. Old leaves are not mottled by the spread and multiplication of the virus. It also was pointed out that one or more leaves that are young at the time of infection may show the symptom described as clearing-of-veins. This symptom frequently occurs in the base of a leaf only. In that case the tip retains its normal green color and is apparently unaffected.

It was of interest to determine whether immunity from the virus of aucuba mosaic would be confined to mottled leaves or would extend to unaffected leaves or leaves with clearing-of-veins.

Each of 10 healthy young plants was inoculated by rubbing the virus of tobacco mosaic over the upper surface of a single leaf. Ten similar plants were left uninoculated and served as checks. Two weeks after inoculation, when one or more of the mottled leaves on each inoculated plant had reached maturity, all full-grown leaves were tagged with labels indi-

eating whether they were mottled, apparently healthy, or whether they showed clearing-of-veins. All leaves that were only partly affected by the clearing-of-veins symptom were marked with dotted lines to indicate exactly what portions were unaffected. The lines were drawn with India ink applied with a camel-hair brush. They were drawn across the upper surfaces of the leaves in such a way as to mark off the portions that were unaffected from those showing chlorosis. Since there is usually a zone of slightly discolored tissue between the areas with marked clearing-of-veins and those of a normal green color, it was not always easy to determine where the line separating affected from nonaffected parts should be drawn. All mature leaves of the 10 plants were heavily inoculated with the virus of aucuba mosaic. Similar leaves on 5 of the check plants were likewise inoculated. The remaining 5 check plants were again left uninoculated.



FIG. 4. Two leaves of *N. sylvestris* partly invaded by the virus of tobacco mosaic as judged by the clearing-of-veins symptom. The invaded portions are immune from aucuba mosaic as is shown by the failure of lesions to develop in these portions.

When the plants were observed 5 days after these inoculations, it was found that numerous necrotic spots, such as are typical of the primary lesions of aucuba mosaic on leaves of *Nicotiana sylvestris*, had developed on all the inoculated leaves of the 5 check plants and on all the normal green leaves of the 10 plants having tobacco mosaic. All the mottled leaves, as well as all leaves showing the clearing-of-veins symptom throughout, were free of lesions. The 5 uninoculated check plants were likewise free of lesions. The leaves bearing India ink lines that marked off areas with clearing-of-veins from areas unaffected by the tobacco mosaic showed practically no lesions in the chlorotic areas below the lines but numerous lesions in the normal green areas above the lines. A few lesions occurred on, or slightly below, the lines in some of the leaves. Since the entire upper surfaces of the leaves were inoculated equally with the virus of aucuba mosaic, it is evident that only those portions visibly affected by tobacco mosaic become immune from aucuba mosaic during the first 2 weeks following inoculation. In several of the leaves, however, narrow zones of lesion-free areas just above the ink lines were observed. Two leaves inoculated with the virus of aucuba mosaic after they had been partly invaded by tobacco mosaic are shown in figure 4. The leaf on the left bears one necrotic lesion just below the inked line. It is probable that the line was incorrectly drawn at the point where the lesion occurs.

Several experiments were made for the purpose of determining whether old leaves that remain entirely free of symptoms acquire immunity from aucuba mosaic. At various intervals after the first appearance of symptoms in young plants, leaves too old to show clearing-of-veins were inoculated by rubbing their upper surfaces with the virus of aucuba mosaic. It any symptoms of tobacco mosaic, become immune from aucuba mosaic. was found that old leaves that remain deep green in color and never show Immunity extends far beyond the parts visibly affected by tobacco mosaic. It is, however, acquired very slowly by the old leaves. Parts of some of them remain susceptible over a period of one or two months following inoculation with tobacco-mosaic virus. Numerous tests have shown that the virus of tobacco mosaic moves into old leaves very slowly. Such leaves acquire immunity from aucuba mosaic soon after being invaded by the virus of tobacco mosaic. The experiments prove that immunity from the former is closely associated with invasion by the latter.

#### TIME REQUIRED FOR THE DEVELOPMENT OF IMMUNITY FOLLOWING SYSTEMIC INFECTION

An experiment was made for the purpose of determining the time required for the development of immunity in young leaves invaded in the course of systemic infection.

Each of 21 healthy young plants was inoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of a single leaf. The plants were then separated into 7 groups of 3 plants each. After one day, and on each successive day over a period of a week, one group of plants was reinoculated by rubbing juice containing the virus of aucuba mosaic over the upper surface of all leaves except those previously inoculated with tobacco-mosaic virus and the young leaves less than 2 in. long. The plants were examined 5 days after the last group was inoculated.

Numerous necrotic lesions appeared on all parts of the leaves of the plants in the first and second groups. In the third group half of a leaf on one plant and about one-third of each of 2 leaves on the other 2 plants were free of lesions. Parts of one or more leaves on each of the plants of the fourth group were likewise free of lesions. In the fifth group large parts of leaves on each of the 3 plants were entirely free of lesions, and one leaf on one plant was lesion-free. One or more leaves on each plant in the sixth and seventh groups were also entirely free of lesions.

The experiment shows that no protection whatever is afforded by systemic infection during the first 2 days following inoculation. Parts of several leaves were protected on the third and fourth days. Not until the fifth day following inoculation was any leaf wholly protected.

The movement of tobacco mosaic virus in *Nicotiana sylvestris* plants may be accurately followed by means of immunity tests. Small quantities of virus do not confer immunity, but the tissues become immune soon after invasion.

#### TIME REQUIRED FOR THE DEVELOPMENT OF IMMUNITY IN LEAVES FOLLOWING DIRECT INOCULATION

It was found that very little protection is afforded by the inoculation of healthy leaves of *Nicotiana sylvestris* immediately before their exposure to aucuba mosaic. An experiment was undertaken for the purpose of determining the time necessary for the development of immunity.

Twenty-four healthy young plants were divided into 8 groups of 3 plants each. One mature leaf on each plant was inoculated by rubbing fresh juice containing the virus of tobacco mosaic over the entire upper surface. A similar leaf on each plant was rubbed with water, it having been shown to be immaterial whether the uninoculated leaves are rubbed with water or with juice from healthy plants. Immediately after this inoculation and on each succeeding day for the following 7 days one group of plants was inoculated by rubbing juice containing the virus of aucuba mosaic over the upper surface of each leaf previously rubbed with juice containing the virus of tobacco mosaic or with water. Four days after the last group was inoculated, all plants were carefully observed. A count or an estimate of the

number of lesions that developed on each leaf was made. The results obtained are recorded in table 1. The table shows that little protection is afforded by rubbing the leaves with virus of tobacco mosaic immediately preceding their inoculation with the virus of aucuba mosaic. After an interval of one day, however, more than twice as many lesions developed in the unprotected as in the protected leaves. After a period of 2 days or

TABLE 1.—*Time required for development of immunity in the leaves of Nicotiana sylvestris following direct inoculation.*

Interval in days between inoculation with viruses of tobacco mosaic and aucuba mosaic	Plant	Number of lesions on leaves rubbed with tobacco-mosaic virus before inoculation with virus of aucuba mosaic	Number of lesions on leaves rubbed with water before inoculation with aucuba-mosaic virus
0	1	239	406
	2	500 <sup>a</sup>	560 <sup>a</sup>
	3	350 <sup>a</sup>	450 <sup>a</sup>
1	4	42	275 <sup>a</sup>
	5	125 <sup>a</sup>	325 <sup>a</sup>
	6	150 <sup>a</sup>	400 <sup>a</sup>
2	7	0	325 <sup>a</sup>
	8	4	400 <sup>a</sup>
	9	9	350 <sup>a</sup>
3	10	6	325 <sup>a</sup>
	11	5	356
	12	5	300 <sup>a</sup>
4	13	1	526
	14	3	450 <sup>a</sup>
	15	0	350 <sup>a</sup>
5	16	0	300
	17	0	350 <sup>a</sup>
	18	0	425 <sup>a</sup>
6	19	0	325 <sup>a</sup>
	20	6	350 <sup>a</sup>
	21		
7	22	0	250 <sup>a</sup>
	23	0	375 <sup>a</sup>
	24	10	225 <sup>a</sup>

<sup>a</sup> Number estimated.

longer few or no lesions developed in the protected leaves. The distribution of the few that did appear was such as to suggest that they developed in areas that escaped inoculation with tobacco-mosaic virus. They were located on the bases or edges of the leaves. Other parts of the leaves were entirely free of lesions. The experiment proves that two days are sufficient

for the development of immunity in leaves directly inoculated with the virus of tobacco mosaic.

#### IMMUNITY CLOSELY CONFINED TO AREAS INOCULATED

The peculiar distribution of the lesions of aucuba mosaic on leaves rubbed with the virus of tobacco mosaic suggested that an experiment should be made to determine whether immunity is closely confined to inoculated leaf areas or may extend somewhat beyond them. The following experiment was undertaken.

Five mature leaves on each of 5 healthy *Nicotiana sylvestris* plants were selected for inoculation. Juice containing tobacco-mosaic virus was rubbed over the upper surface of one side of each leaf. The upper surface of the other side was rubbed with juice from a healthy plant. An effort was made to rub the 2 sides equally, so that trichomes and upper epidermal cells on both sides would be wounded to approximately the same extent. Three days after this inoculation the leaves were given a second inoculation by rubbing juice containing the virus of aucuba mosaic over their entire upper surfaces. Four days after this second inoculation it was found that an average of about 200 necrotic spots, typical of the primary lesions of aucuba mosaic, had developed on the sides of the leaves previously rubbed with virus-free juice, whereas an average of only 12 necrotic spots had developed on the sides rubbed with juice containing tobacco-mosaic virus. On 5 of the leaves no lesions whatever occurred on the sides inoculated with tobacco mosaic. The lesions were rather evenly distributed over the sides rubbed with virus-free juice, but very unevenly distributed over the sides rubbed with tobacco-mosaic virus. Most of the lesions were confined to the edges and bases of these sides. Such areas may have escaped inoculation with the tobacco-mosaic virus. There was no indication that inoculation of half of a leaf with tobacco-mosaic virus affects in the slightest degree the susceptibility of the other half to the virus of aucuba mosaic over the period tested. The experiment proves that leaf areas inoculated with the virus of tobacco mosaic acquire immunity from the virus of aucuba mosaic, but that adjacent areas not so inoculated remain susceptible. Immunity does not extend much beyond the areas inoculated.

#### HEAVY INOCULATION NECESSARY FOR THE COMPLETE IMMUNIZATION OF MATURE LEAVES

In the experiment described above, only 5 of the leaves remained entirely free of necrotic lesions on the sides rubbed with tobacco-mosaic virus. This suggested that a very thorough inoculation with tobacco-mosaic virus may be necessary to obtain complete protection against aucuba mosaic. An attempt was made to inoculate certain parts of leaves more completely and

more heavily with the tobacco-mosaic virus than was done in the foregoing experiments.

Forty-six mature leaves on 8 healthy young plants of *Nicotiana sylvestris* were inoculated with the virus of tobacco mosaic in the following manner. The upper surface of one side of each of 6 leaves on each of 2 plants was very thoroughly and evenly rubbed with fresh juice containing the virus of tobacco mosaic. Care was taken to avoid missing any part of the leaf surface on the side being inoculated. The upper surface of the tip portion of each of 4 leaves on one plant, 6 leaves on another, and 7 leaves on a third, was thoroughly rubbed with another sample of the same inoculum. The upper surface of the basal portion of 5 leaves on each of 2 plants, and of 7 leaves on another plant, was similarly inoculated. The upper surfaces of the uninoculated portions of each of the leaves were just as thoroughly rubbed with water.

Five days after inoculation with the virus of tobacco mosaic, all leaves were given a second inoculation by rubbing the virus of aucuba mosaic over their entire upper surfaces. All portions of the leaves were inoculated equally with virus from the same sample of juice.

Three days after the second inoculation all leaves were carefully observed. The 12 inoculated with tobacco-mosaic virus on one side only were entirely free of necrotic lesions on the inoculated sides. From 125 to 500 necrotic spots typical of the primary lesions of aucuba mosaic were present on the opposite sides. A careful estimate showed that about 3250 lesions had developed on the sides of the leaves not inoculated with tobacco-mosaic virus. The sides heavily inoculated were completely protected against aucuba mosaic.

Of the 17 leaves inoculated with tobacco-mosaic virus in their tips, 12 showed no necrotic lesions in the areas so inoculated. Two showed 1 lesion each, and one showed 2, one 4, and one 5 lesions in their tip areas. Necrotic lesions were well distributed over the basal portions of all of the 17 leaves. A count showed that there were about 2730 lesions on the basal portions of the 17 leaves.

Of the 17 leaves inoculated with tobacco-mosaic virus over their basal portions, 12 were entirely free of lesions in these halves. One showed 1 lesion, one 2 lesions, and three 5 lesions each, in the inoculated areas. Necrotic lesions were well distributed over the tip portions of all leaves. It was estimated that more than 5000 lesions were present on the tip halves of the 17 leaves.

A total of 31 necrotic lesions of aucuba mosaic developed in the protected areas of 10 leaves. No lesions whatever developed in the protected areas of the other 36 leaves. Approximately 11,000 lesions appeared in the unprotected halves of the 46 leaves. The experiment shows how fully





FIG. 5. Three leaves of *N. sylvestris* partly immunized from aucuba mosaic by direct inoculation with tobacco mosaic. The areas free of lesions were rubbed with juice containing the virus of tobacco mosaic 5 days before all parts of the leaves were inoculated equally with aucuba mosaic.

leaves, or portions of leaves, may be protected against aucuba mosaic by heavy inoculation with the virus of tobacco mosaic, and how closely protection is confined to the areas inoculated. A leaf taken from each set of plants used in this experiment is shown in figure 5. The left half of the leaf on the left, the lower half of the middle leaf, and the upper half of the leaf on the right were inoculated with the virus of tobacco mosaic 5 days before all parts of the upper surfaces of all leaves were inoculated equally with the virus of aucuba mosaic.

#### LOCAL PROTECTION NOT CONFINED TO CELLS ACTUALLY INOCULATED WITH TOBACCO-MOSAIC VIRUS

It is evident from the foregoing experiments that in mature leaves protection is rather closely confined to the areas inoculated. The following experiment was undertaken for the purpose of determining whether inoculation of the lower surfaces of mature leaf areas with tobacco-mosaic virus would protect such areas against subsequent infection with the virus of aucuba mosaic when inoculated into the upper surfaces.

Two mature leaves on each of 10 healthy young plants were inoculated by rubbing juice containing the virus of tobacco mosaic over the lower surface of one side of each leaf. The lower surface of the other side was in each case rubbed with water. Two similar leaves on each of the 10 plants

were inoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of one side of each leaf. The upper surface of the other side of each leaf was similarly rubbed with water.

Four days after these inoculations, all of the leaves were inoculated a second time by rubbing fresh juice containing the virus of aucuba mosaic over their entire upper surfaces.

Five days after the second inoculation, the leaves were carefully observed. There was very little difference to be seen between the degree of protection obtained by inoculation of the lower surfaces, and that obtained by inoculation of the upper surfaces. The protection afforded by both methods was almost, but not quite, complete. A few lesions developed on the edges of the protected halves of most of the leaves. The experiment proves that protection extends somewhat beyond the cells actually inoculated. A microscopic examination of sections through the leaves showed them to have a minimum thickness of 7 cell layers. Five layers of cells intervened between the cells of the lower epidermis that were inoculated and those of the upper epidermis that were protected.

#### LITTLE OR NO MULTIPLICATION OF AUCUBA-MOSAIC VIRUS IN PROTECTED TISSUES

In the experiments described up to this point, no attempt was made to determine whether the protection due to direct inoculation of leaves with tobacco-mosaic virus consists in preventing infection by the virus of aucuba mosaic, or only in inhibiting the symptoms of this disease. An experiment was, therefore, made to determine whether the virus of aucuba mosaic multiplies in protected tissues.

Two mature leaves on each of 6 healthy young plants were inoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of one side of each leaf. The other sides of the leaves were rubbed with water. Five days after this inoculation, all of the leaves were given a second inoculation by rubbing their entire upper surfaces with juice containing the virus of aucuba mosaic. When the leaves were observed 3 days after the second inoculation, it was found that numerous necrotic spots typical of the primary lesions of aucuba mosaic had developed on all of the leaves on the unprotected sides, whereas no lesions had developed on the protected sides. Two discs of tissue about 1 cm. in diameter were cut from each side of each of the 12 leaves by means of a sterile cork borer. Each disc was crushed in a few drops of water on a glass slide. The juice obtained from each of the 24 discs of unprotected tissue was used to inoculate one side of each of 24 mature leaves on 6 healthy *Nicotiana sylvestris* plants. The opposite sides of the leaves were inoculated with juice obtained from each of the 24 discs of protected tissue. The leaves were

observed 4 days after inoculation. A total of 414 lesions were counted on the sides inoculated with juice from unprotected tissue, but only 6 were found on the sides inoculated with juice from protected tissue. The experiment shows that little or no multiplication occurs in the protected tissues. The 6 lesions obtained by inoculating with juice from discs of protected tissue may have resulted from virus left on these discs when they were inoculated with aucuba mosaic.

#### THE IMMUNE REACTION IN PLANTS OF DIFFERENT AGES

An experiment was made to determine whether the leaves of old plants are as easily protected against aucuba mosaic as those of young ones. Nine healthy *Nicotiana sylvestris* plants 57 days old, nine 109 days old, and nine 165 days old were divided into 3 groups of 9 plants each. Three plants of each of the 3 different ages were placed in each group. The plants of the first group were inoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of one side of each leaf large enough to inoculate; those of the second group by rubbing a like sample of juice over the upper surface of the tip portion of each leaf; and those of the third group by rubbing a similar inoculum over the upper surface of the basal portion of each leaf. The uninoculated parts of all leaves were rubbed with water. Three days after their inoculation with tobacco-mosaic virus



FIG. 6. Three leaves of *N. sylvestris* from plants 5½ months old. The areas bearing few lesions were rubbed with juice containing the virus of tobacco mosaic 3 days before all parts of the leaves were inoculated equally with aucuba mosaic.

all leaves were inoculated a second time by rubbing juice containing the virus of aucuba mosaic over their entire upper surfaces.

When the plants were observed 5 days after the second inoculation it was found that numerous necrotic lesions had developed on all of the unprotected portions of the leaves. A small number of lesions had also developed in the protected areas of many leaves. The leaves of the youngest plants were more completely protected than those of the other 2 sets. The best protection was obtained in the oldest leaves of the youngest plants. The poorest protection was obtained in leaves of the oldest plants, but, even in these, it was quite marked, as may be seen from figure 6. The picture shows a leaf from one of the oldest plants in each of the 3 groups. The experiment proves that inoculation with tobacco-mosaic virus affords local protection against aucuba mosaic in old as well as in young plants.

#### RECIPROCAL CROSS-IMMUNITY TESTS

A number of reciprocal cross-immunity tests were undertaken. The object of the tests was to determine whether inoculation of leaves with the virus of aucuba mosaic would render them immune from tobacco mosaic.

In a typical experiment, 3 leaves on each of 10 healthy young plants were inoculated with aucuba mosaic by means of needles. The needle-puncture method was used in preference to the rubbing method, because the number of points of infection is more easily controlled when the latter method is used. Each leaf received 100 punctures with needles dipped in juice containing the virus. On the fourth day following this inoculation, when necrotic lesions resulting from inoculations by the needle punctures had reached a diameter of  $\frac{1}{4}$  cm., the leaves were reinoculated by rubbing one side of the upper surface of each with juice containing the virus of tobacco mosaic. The plants were held under observation for 3 weeks following this inoculation. The sides of the leaves inoculated with tobacco mosaic yellowed somewhat as a result of the development of numerous primary lesions at the points of infection with tobacco mosaic. The plants all came down with systemic infection. There was at no time any visible evidence that tissues adjacent to aucuba mosaic lesions had developed immunity from tobacco mosaic. The lesions produced by the latter were, however, too indistinct to be differentiated with certainty from yellowing due to other causes. Yellowing associated with the killing action of aucuba mosaic in tissues surrounding the lesions interferes with observations on the tobacco-mosaic lesions. The technique is inadequate for the purpose of demonstrating immunity in tissues surrounding these lesions. It is possible that suitable staining methods might show immunity. All that this and other similar experiments prove is that small numbers of aucuba mosaic lesions do not render leaves immune from tobacco mosaic.

IMMUNIZATION BY INOCULATION WITH ATTENUATED STRAINS OF  
TOBACCO MOSAIC

Following the method described by Johnson (12) for the attenuation of tobacco mosaic by heat, 3 attenuated strains of this virus were secured. They were isolated from 3 different tobacco plants incubated at about 35° C. for 15 days after being inoculated with tobacco mosaic. The 3 strains showed different degrees of attenuation as judged by the symptoms produced on tobacco and other plants. All caused milder symptoms than the virus from which they were obtained. The strain selected for use in the experiments to be reported produced a mild type of mottling and did not cause much stunting in either tobacco or *Nicotiana sylvestris* plants. Experiments were made to determine whether this attenuated strain would give protection against aucuba mosaic.

Ten healthy young plants were inoculated with tobacco mosaic. Ten similar plants were inoculated with the attenuated virus, and 10 were left uninoculated. After a period of 6 weeks, the plants inoculated with attenuated virus showed a mild type of mottling and were somewhat stunted in growth. They were, however, much larger than the plants inoculated with tobacco mosaic. Two of the youngest mature leaves of each of the 30 plants were then inoculated by rubbing fresh juice containing the virus of aucuba mosaic over their entire upper surfaces. Five days after this inoculation it was found that numerous necrotic spots typical of the primary lesions of aucuba mosaic had developed on each of the inoculated leaves of the 10 check plants. No lesions had developed on any of the leaves of the 10 plants inoculated with tobacco mosaic nor on the leaves of the 10 plants inoculated with the attenuated strain of this virus. Figure 7 shows a leaf from a healthy plant and one from a plant infected with attenuated virus, 5 days after both were inoculated equally with aucuba mosaic. The experiment proves that *Nicotiana sylvestris* leaves mottled by this attenuated virus are as completely protected against aucuba mosaic as similar leaves mottled by ordinary tobacco mosaic.

In another experiment, one side of one leaf on each of 10 healthy young *Nicotiana sylvestris* plants was heavily inoculated by rubbing juice containing the virus of tobacco mosaic over its upper surface. A similar leaf on each plant was inoculated by rubbing juice containing the attenuated virus over the upper surface of one side. The upper surfaces of the uninoculated sides of all of the leaves were rubbed with water. Five days after this inoculation all leaves were inoculated a second time by rubbing fresh juice containing the virus of aucuba mosaic over their entire upper surfaces. When the plants were observed on the sixth day following the second inoculation, it was found that numerous necrotic lesions of aucuba mosaic had



FIG. 7. Two leaves from *N. sylvestris* plants 5 days after both were inoculated equally with aucuba mosaic. The leaf on the left is from a plant infected with an attenuated strain of tobacco mosaic; that on the right from a healthy plant.

developed on the sides of the leaves rubbed with water, whereas very few lesions had developed on the sides inoculated with either tobacco-mosaic virus or with the attenuated strain of this virus. The sides of two of the leaves inoculated with tobacco-mosaic virus showed 5 lesions each. The corresponding sides of the other 8 leaves were free of lesions. The sides of 4 leaves inoculated with attenuated virus showed 1, 2, 4, and 5 lesions respectively. The corresponding sides of the other 6 leaves were free of lesions. A total of only 10 lesions appeared on the sides of the leaves inoculated with tobacco-mosaic virus. The opposite unprotected sides were estimated to bear about 3000 lesions. A total of 12 lesions appeared on the sides of the 10 leaves inoculated with attenuated virus. The opposite unprotected sides of the same leaves were estimated to bear about 2800 lesions.

The experiment brings evidence that even in mature leaves this attenuated strain of virus gives protection against aucuba mosaic equal to that afforded by ordinary tobacco mosaic. Similar tests of the other 2 attenuated strains gave evidence that they also afford the same protection.

#### PROTECTION OF PLANTS AGAINST TOBACCO MOSAIC BY ATTENUATED VIRUS

An experiment was made for the purpose of determining whether the immunity conferred by attenuated strains of tobacco mosaic would protect plants against tobacco as well as aucuba mosaic.

Twenty healthy young plants were inoculated with an attenuated strain of the virus of tobacco mosaic. Twenty similar plants left uninoculated served as checks. When, after a period of one week, the inoculated plants were showing early symptoms of infection, 10 of them were reinoculated by rubbing juice containing the virus of tobacco mosaic over the upper surface of the first leaf above that into which attenuated virus was rubbed. Ten of the healthy plants were similarly inoculated. All of the plants were held under observation for one month after the tobacco mosaic inoculations were made. The 10 uninoculated check plants remained healthy. The 10 plants inoculated with attenuated virus, only, came down with the symptoms typical of the disease produced by this virus. The leaves were somewhat mottled but not distorted. The plants were slightly stunted. The 10 plants inoculated with tobacco mosaic only, came down with tobacco mosaic. On the fifth day following inoculation, one or more leaves on each plant showed the clearing-of-veins symptom. This was followed by mottling and distortion of leaves. The plants were badly stunted when the experiment was ended. The 10 plants inoculated with tobacco-mosaic virus one week after being inoculated with attenuated virus, came down with symptoms characteristic of the disease caused by the latter. At no time did any of them show the symptoms of tobacco mosaic. The leaves were not severely mottled and the plants only slightly stunted. These plants were, in fact, indistinguishable from those inoculated with attenuated virus only. The experiment proves that attenuated virus may be used for the protection of plants against tobacco mosaic.

#### SPECIFICITY OF THE IMMUNE REACTION

In order to secure evidence as to the specificity of protection by inoculation of plants or individual leaves with tobacco-mosaic virus or its attenuated strains, experiments were made for the purpose of determining whether cucumber mosaic or the ring-spot of tobacco might give protection against aucuba mosaic.

Each of 10 plants that had shown the symptoms of cucumber mosaic for

about 5 weeks was inoculated by rubbing juice containing the virus of aucuba mosaic over the upper surface of 2 full-grown mottled leaves. Ten other plants that had had tobacco mosaic for a like period of time were similarly inoculated. Two leaves on each of 10 healthy plants were likewise inoculated and served as checks. When the leaves were examined 5 days after these inoculations it was found that necrotic lesions had developed on all of the check leaves and on all of the leaves mottled by cucumber mosaic. No lesions whatever had developed on the leaves mottled by tobacco mosaic. A typical leaf from one of the plants having cucumber mosaic is shown in figure 1, B. The lesions of aucuba mosaic occur in the light green, as well as in the dark green, areas of the leaf. The experiment proves that the systemic infection of leaves by cucumber mosaic does not protect them against aucuba mosaic.

As is well known from the work of Price (17), *Nicotiana sylvestris* plants regularly recover and are subsequently immune from ring-spot of tobacco. An experiment was made for the purpose of determining the reaction of recovered plants to aucuba mosaic.

Each of 10 plants that had suffered an attack of ring spot and had recovered to such an extent that their tips, including several mature leaves, appeared to be healthy were inoculated by rubbing fresh juice containing the virus of aucuba mosaic over the upper surface of 2 of the youngest mature leaves. Two mottled leaves on each of 10 plants having tobacco mosaic were similarly inoculated. Two leaves on each of 10 healthy plants inoculated in the same manner served as checks.

On the fifth day following these inoculations, it was found that numerous necrotic lesions had developed on all of the check leaves. An approximately equal number had developed on the inoculated leaves of the plants that had recovered from ring spot. No lesions whatever appeared on the leaves mottled by tobacco mosaic. The experiment proves that leaves of plants that have recovered from ring spot and are immune from this disease are not thereby protected against aucuba mosaic, although, under the conditions of the experiment, similar plants having tobacco mosaic are fully protected against this disease.

An experiment was made for the purpose of determining whether mature leaves of *Nicotiana sylvestris* can be protected against aucuba mosaic by direct inoculation with either the virus of cucumber mosaic or that of ring spot.

A mature leaf on each of 10 healthy young plants was inoculated by rubbing juice containing the virus of cucumber mosaic over its entire upper surface. A second, third, and fourth leaf on each plant was rubbed with juice containing the virus of ring spot, juice containing the virus of tobacco mosaic, and water, respectively. Five days after these inoculations all of the leaves were rubbed with juice containing the virus of aucuba mosaic.



On the fourth day following this inoculation all leaves rubbed with water, with juice containing the virus of cucumber mosaic, or with juice containing the virus of ring spot, were observed bearing numerous necrotic spots typical of the primary lesions of aucuba mosaic. The leaves rubbed with juice containing the cucumber-mosaic virus or the ring-spot virus bore approximately the same number of lesions as those rubbed with water. Three of the 10 leaves rubbed with tobacco-mosaic virus bore no lesions whatever. The other 7 leaves bore a few scattered lesions on their edges. The experiment proves that no protection whatever is afforded against aucuba mosaic by the direct inoculation of leaves with the virus of either cucumber mosaic or ring spot under conditions favorable for the development of protection when tobacco mosaic is used.

During the course of further studies on the possibility of protecting leaves by inoculating them with these viruses, it was observed that lesions of ring spot retard the development of the primary lesions of aucuba mosaic. When a leaf bearing a small number of ring-spot lesions is inoculated by rubbing the upper surface with juice containing the virus of aucuba mosaic, lesions of the latter appear in the green portions of lesions of the former. Such lesions develop more slowly than those in other parts of the same leaf.

#### DISCUSSION

The experiments reported in this paper show that the leaves of *Nicotiana sylvestris* plants may be immunized against aucuba mosaic by inoculation with tobacco mosaic or its attenuated strains. They may also be immunized by inoculation with attenuated strains of aucuba mosaic. The very conspicuous primary lesions produced by aucuba mosaic give a convenient and accurate test that makes possible observations on the rate of development, extent, and specificity of the immune reaction.

The leaves of healthy *Nicotiana sylvestris* plants are highly susceptible to infection by the virus of aucuba mosaic. The inoculation of such plants with tobacco-mosaic virus does not immediately alter their susceptibility to aucuba mosaic. A definite period of time must elapse before plants inoculated with the virus of tobacco mosaic acquire immunity from aucuba mosaic. It is evident that immunity develops after, and as a result of, tobacco-mosaic infection. The infection of any given leaf by the virus of tobacco mosaic may result from direct inoculation of the leaf, or from invasion in the course of systemic infection. If infection is by direct inoculation, the areas immunized coincide very closely with the areas inoculated. If complete immunization is desired, the inoculation must be heavy. In that case the interval between inoculation and complete immunization is about 2 days. There is at this time no visible evidence of infection. It is a striking fact that tissues that are highly susceptible become immune within 2 days without undergoing any visible change. If

infection is by systemic invasion, the interval is somewhat longer. It is, however, complete regardless of the extent or degree of severity of inoculation. All leaves in which clearing-of-veins or mottling appears become immune. Leaves or parts of leaves too old to be visibly affected by systemic invasion, acquire immunity very slowly. Even in young plants, such leaves remain susceptible for a month or 6 weeks after the inoculation of the plants. They finally become immune without losing their deep green color. These leaves, however, may be quickly immunized at any time by direct inoculation. The virus of aucuba mosaic does not multiply appreciably in protected cells. This undoubtedly accounts for the failure of lesions to develop in protected areas.

Nothing whatever is known regarding the nature of the process by which leaf tissues are protected against aucuba mosaic. The immune reaction is so closely associated with the spread and multiplication of the virus of tobacco mosaic that it has not been possible to determine whether the inhibiting factor may be the virus or some substance produced in its presence. It is conceivable that closely related viruses multiply in and occupy the same parts of infected cells and that, when these parts are filled by one of them, there is no room for another. It is also conceivable that related viruses make use of the same materials in their multiplication, and that, when the materials are exhausted by one, the multiplication of another is prevented. On the other hand, it is possible that protection may be due to immunizing substances produced by infected cells. It does not seem likely that protection results from morphological changes in the tissues, since immunity is acquired by old as well as by young tissues. The deep green areas of leaves mottled by tobacco mosaic contain less virus than the light green areas. They are, nevertheless, immune from aucuba mosaic. Whatever the factor may be that protects against aucuba mosaic, it doubtless operates in limiting the severity of tobacco mosaic. The fact that this disease does not, under ordinary conditions, kill plants, may be due to a defense mechanism which holds the multiplication of the virus in check. There is at present no satisfactory technique for demonstrating such a mechanism for the virus of tobacco mosaic.

There is evidence that the immunity conferred by tobacco-mosaic virus and its attenuated strains may be specific. Cucumber mosaic gives no protection against aucuba mosaic. Plants that have recovered and are immune from ring-spot are quite susceptible to aucuba mosaic. Much further work must, however, be done before the specific nature of the immune reaction is proved. If the reaction is found to be specific, it will be useful in differentiating related from unrelated viruses.

The immunization of plants against aucuba and tobacco mosaic by means of highly attenuated strains of the latter offers interesting possibilities for the practical control of these diseases. Some attenuated strains

cause such slight injury that their use in the protection of plants might be advisable in situations where the incidence of one or both of these diseases is high. The principles involved are the same as those made use of in human and animal pathology for immunization by vaccination.

#### SUMMARY

Certain symptoms by which tobacco and aucuba mosaics may be distinguished on *Nicotiana sylvestris* are described.

Cross-immunity studies show that plants infected by attenuated strains of the virus of aucuba mosaic become immune from unattenuated virus. Plants infected by tobacco-mosaic virus become immune from aucuba mosaic, except in the youngest leaves.

Mature leaves of healthy plants inoculated with tobacco-mosaic virus acquire immunity from aucuba mosaic in the parts inoculated.

Leaves must be heavily inoculated if complete immunization is desired. The immune reaction is closely confined to the parts inoculated.

Immunity resulting from systemic infection is closely associated with invasion of tissues by the virus of tobacco mosaic.

Immunity resulting from direct inoculation develops within 2 days. That following systemic infection develops more slowly.

The virus of aucuba mosaic multiplies little or none in protected tissues.

Immunity resulting from direct inoculation develops in old as well as in young plants.

Protection by local inoculation is not confined to cells actually inoculated.

Plants inoculated with attenuated strains of the virus of tobacco mosaic become immune from both tobacco and aucuba mosaics.

Due, it is believed, to inadequate technique, reciprocal cross-immunity tests failed to demonstrate immunization by aucuba mosaic.

No protection against aucuba mosaic is afforded by the infection of plants either with cucumber mosaic or with ring spot of tobacco.

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# STUNT—A VIROSIS OF DELPHINIUM<sup>1</sup>

GROVER BURNETT<sup>2</sup>

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## INTRODUCTION

The garden varieties of perennial delphinium in the state of Washington are affected with a virosis, which is readily transmissible by mechanical means to tomato, cucumber, tobacco, and several other species of plants.

A virosis of delphinium closely resembling ring spot of tobacco was reported in 1928 by Vallean.<sup>3</sup> The same year Linford<sup>4</sup> reported yellows of delphinium in Utah. The characteristic symptoms of yellows caused the plants to become chlorotic in advance of blossoming followed by a leafy proliferation of the floral parts. In 1930 Vallean<sup>5</sup> reported a virosis of delphinium, transferable to tobacco, which appeared to be identical with the "coarse etch" of tobacco.

In a more detailed report by Vallean<sup>6</sup> in 1932 he states "The Delphinium virus corresponds most closely to the typical cucumber viruses, . . ."

A disease of delphinium designated as witches' broom by Hungerford<sup>7</sup> was reported in Idaho in 1933. He stated ". . . The appearance and general conditions surrounding the occurrence of this disease would indicate that it is probably due to a virus."

## MATERIALS AND METHODS

The experimental work was carried on in the greenhouse and in the field at the State College of Washington, Pullman, Washington, in 1932 and 1933.

In October, 1932, a number of delphinium plants which appeared to be diseased, as well as some healthy plants, were transplanted into deep boxes in the greenhouse. These plants produced sufficient new foliage within 3

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<sup>2</sup> The writer wishes to acknowledge the valuable assistance, advice, and criticism given by Dr. L. K. Jones and Dr. F. D. Heald during the progress of this investigation.

<sup>3</sup> Vallean, W. D. Ring spot. U. S. Dept. Agr. Bur. Pl. Indus. Pl. Dis. Rptr. Sup. 65: 419. 1927.

<sup>4</sup> Linford, M. B. Yellows. Pl. Dis. Rptr. Sup. 65: 420. 1927.

<sup>5</sup> Vallean, W. D. A virus disease of Delphinium reported from Kentucky. Pl. Dis. Rptr. 14: 118. 1930.

<sup>6</sup> ———. A virus disease of Delphinium and tobacco. Ky. Agr. Exp. Sta. Res. Bul. 327: 81-88. 1932.

<sup>7</sup> Hungerford, C. W. A new virus disease of Delphinium in Idaho. Pl. Dis. Rptr. 17: 5. 1933.

weeks to serve as a source of inoculum. When seedlings of delphinium, tobacco, tomato, cucumber, and several other species of plants were inoculated with the macerated leaf tissue<sup>s</sup> of the naturally infected delphinium plants a virosis became evident on the inoculated plants. Depending on the species of plants inoculated, symptoms became evident in about 4 to 20 days. The virus was recovered on tobacco plants from some of the inoculated species that failed to produce evident symptoms.

All of the species tested and recorded in this report were grown from seed and there was no evidence of seed transmission. The test plants were grown in the greenhouse at temperatures ranging from 65° to 75° F. Most of the plants under observation were grown in 3-inch pots but some that were held to maturity were transplanted to raised benches.

#### TRANSMISSION OF THE DELPHINIUM VIRUS TO DELPHINIUM AND OTHER SUSCEPTIBLE SPECIES

A virus naturally occurring on delphinium in the State of Washington has been experimentally transmitted by mechanical means to 4 varieties of delphinium and 12 other species of plants, including tomato, cucumber, and tobacco (Table 2). The virus was recovered from 12 of the 22 delphinium plants tested (Table 1). Sixteen other species of plants showed no evident symptoms when inoculated with the virus and the virus was not recovered from these plants when transfers were made back to tobacco plants.

The transmission of this virus has been demonstrated by evident symptoms produced on these inoculated hosts and by symptoms produced when inoculations were made on tobacco plants.

TABLE 1.—*Recovery of the virus on tobacco from delphinium naturally infected in the field*

Number of delphinium tested	Number positive	Inoculations to tobacco	
		Number plants inoculated	Number positive
22	12	145	51

#### SYMPTOMS ON DELPHINIUM

The symptoms attributed to this disease on naturally infected delphinium in the garden or nurseries are extremely variable. One of the outstanding symptoms is a stunting and dwarfing (Fig. 1, B) and eventual death of

<sup>s</sup> Jones, L. K. A new method of inoculating with viruses. (Abst.) *Phytopath.* 22: 998-999. 1932.

to each of the above light periods. One set was placed in ordinary light as a control. The plants were subjected to the above treatment for 32 days after inoculation.

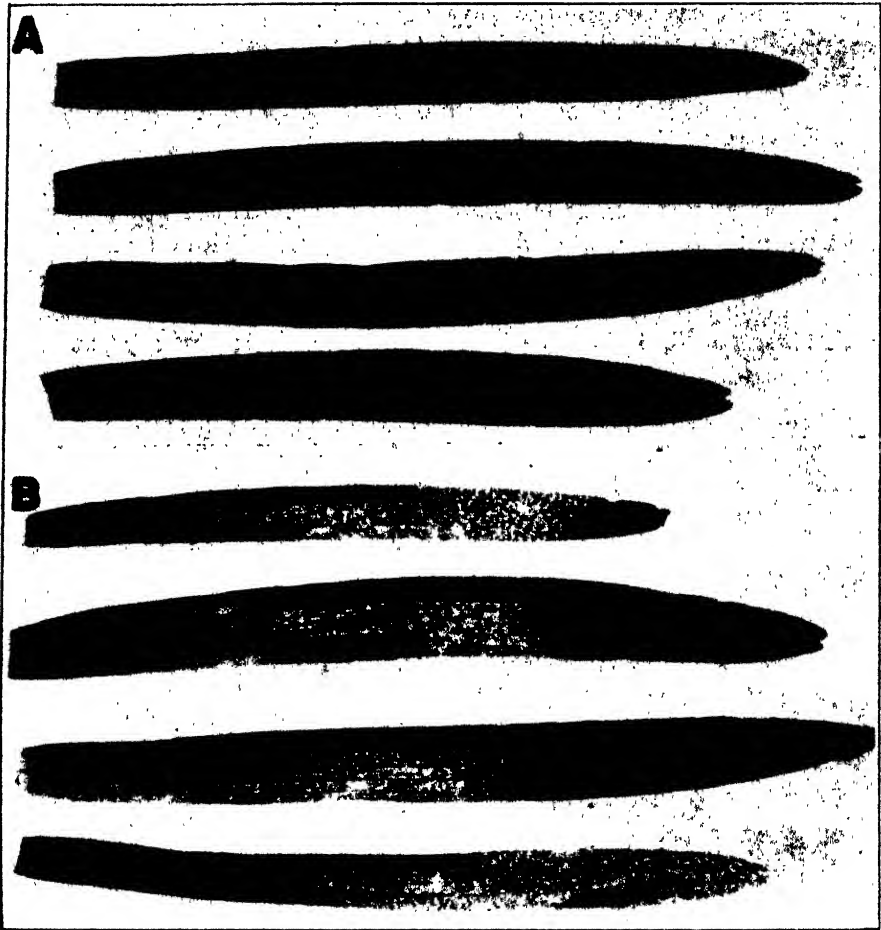
In the various light exposures, supposedly comparable to different day lengths, little difference was observed up to a certain exposure (Figs. 1, 2 and 3 A). There was a direct relationship between the time in which sporulation appeared and the length of exposure. This relationship also held true in the subsequent development of the rust. With 6 hours of light per day, the rust was considerably slower, both in sporulation and development, than that of the 8-hour day and so on, up to the 12-hour day. More than 12 hours of light per day gave a distinct change in rust type, as shown in table 1 and figure 3 A.

TABLE 1.—*The effect of different day lengths on the length of incubation period and type of infection produced by Puccinia glumarum on Pannier barley, C. I. No. 1330*

Temperature			Day length	Period of incubation	Plants inoculated	Plants infected	Type of infection*
Min.	Max.	Mean					
° F.			hrs.	days			
45	50	47.5	6	20	25	21	4
45	50	47.5	8	16	25	20	4
45	50	47.5	10	13	25	24	4
45	50	47.5	12	9-11	25	24	4
45	50	47.5	15	12	25	19	0
45	50	47.5	continuous	12	25	15	0
50	55	52.5	ordinary day	21	25	3	4

\* 4 = completely susceptible; 3 = moderately susceptible; 2 = moderately resistant; 1 = very resistant; 0 = extremely resistant; I (or) — = immune.

It will be noticed from the above table that, up to a certain point, the day length does not affect the type of infection. It does, however, change the length of the incubation period considerably and the subsequent development of the rust (figs. 1, 2). A 6-hour day, in comparison with the 12-hour day, lengthens the period of incubation 9 days. As the day length increases, the period of incubation decreases up to a certain point. There is essentially no difference in the length of the incubation period when the day length is 12 hours or longer. It also will be noticed that a day length greater than 12 hours changes the type of infection from a type-4 to a type-0. In the case of the ordinary day it will be noted that the temperature was a little higher and the period of incubation longer than in the other part of the experiment. The higher temperature resulted from failure to



• FIG. 1. Thirty-two-day-old cultures of *Puccinia glumarum* on leaves of barley plants exposed at 45–50° F. to light for six hours per day (A) and for eight hours per day (B).

control the temperature as accurately in the greenhouse as in the temperature-control room. The longer incubation period in case of the ordinary day was caused by the lowered light intensity of the cloudy days of the winter months while the work was in progress. The writer has often found it difficult to propagate *Puccinia glumarum* in the greenhouse during winters of little sunshine, while in winters of considerable sunshine it can be easily propagated. This fact substantiates the data given in table 1. The above experiment was repeated at a temperature range of 55 to 60° F. with essentially no difference in results. At temperatures of 68 to 70° it was found necessary to keep bell jars over the plants and the soil in the pots





FIG. 2 Thirty two day old cultures of *Puccinia glumarum* on leaves of a barley plant exposed at 45-50° F to light for 10 hours per day (A) and twelve hours (B).

thoroughly wet to secure any sporulation at all. Under these conditions a type-4 infection was obtained. At temperatures above 70° very little infection developed and that little belong either to type 2 or 3. At a temperature of 80° no infection was secured. These results accord essentially with those obtained by Newton, Johnson, and Brown (5) in their studies on stripe rust in Canada.

No attempts have been made definitely to explain this phenomenon. Garner and Allard (2), however, have found in their study of other types of plants that a day length of over 12 hours has a tendency to change the nitrogen-carbohydrate ratio in the plant. In other words, there is a translocation of plant constituents. On this basis it was thought that possibly the same might hold true in the case of *Hordeum vulgare* (L.) and, hence,

the change in resistance of the plant. It is a well-known fact that the rust fungus is primarily a carbohydrate feeder; therefore, any treatment that would cause a change in the nitrogen-carbohydrate ratio in the host plant might create an apparent resistance to the parasite that would not otherwise occur. This explanation as a probable cause of the change in rust type cannot be considered conclusive. Further experimentation is necessary and a complete chemical analysis of the plants must be made before any definite conclusion can be drawn. Another possible explanation of this phenomenon is the fact that a long period of light may have a lethal effect on the rust mycelium in the tissue. This is easily conceivable because the leaf tissue of barley seedlings is very thin, allowing considerable light to pass through. One must bear in mind, however, that the effect of the light is lethal only to the extent of inhibiting sporulation. The rust mycelium continued to grow during the entire period of the experiment but produced no spores.

#### LIGHT INTENSITY STUDIES

In these studies the same general procedure was followed as that described in the foregoing discussion. Each of a set of 5 pots containing the inoculated plants was subjected to a different light intensity. One set of inoculated plants was exposed to 960 foot candles of light supplied by a 1000-watt lamp; the second set was exposed to 576 foot candles of light supplied by 3 200-watt lamps; the third set to 384 foot candles of light supplied by 2 200-watt lamps; the fourth to 192 foot candles supplied by a 200-watt lamp and the fifth set to 96 foot candles supplied by a 100-watt lamp. When more than one lamp was used to supply the necessary intensity, they were so slanted that the resulting light was concentrated into a circle. The plants were exposed to light 9 hours daily.

\* TABLE 2.—*The effect of light intensity on the subsequent development of Puccinia glumarum on Pannier barley*

Temperature Min. Max. Mean			Light intensity		Incubation  Days	Plants inoculated	Plants infected	Type of infection
° F.			Watts	Foot candles				
45	50	47.5	1000	960	11	25	22	4
45	50	47.5	600	576	12	25	25	4
45	50	47.5	400	384	13	25	20	4
45	50	47.5	200	192	13	25	20	4
45	50	47.5	100	96	16	25	18	4
50	55	52.5	ordinary day		20	25	24	4

TABLE 2.—Results of inoculations to various species of plants with delphinium virus

Species of plants inoculated	Number inoculated	Number positive	Inoculations back to tobacco	
			Number inoculated	Number positive
<i>Delphinium</i> sp.				
Chimes Blue	15	5	115	19
White Butterfly	15	9	115	29
Wrexham	10	1	70	9
Common Belladonna type	15	8	115	38
<i>Nicotiana tabacum</i>	53	51		
<i>Lycopersicum esculentum</i>	63	52	64	51
<i>Nicandra physalodes</i>	19	19	25	25
<i>Solanum nigrum</i>	12	5	6	4
<i>Stellaria media</i>	11	8	10	10
<i>Lactuca scariola</i>	5	3	17	11
<i>Cucumis sativus</i> (cucumber)	12	7	3	3
<i>Zinnia</i> sp.	5	2	2	2
<i>Marrubium vulgare</i>	4	2	7	5
<i>Anthemis cotula</i>	3	1	13	6
<i>Capsella bursa-pastoris</i>	5	3	13	13
<i>Petunia hybridum</i>	5	1	2	1
<i>Solanum tuberosum</i>				
Early Rose—virus free	4	0	30	0
Seedling—virus free	19	0	65	0
<i>Datura stramonium</i>	24	0	68	0
<i>Solanum pseudocapsicum</i>	5	0	5	0
<i>Chenopodium album</i>	6	0	17	0
<i>Pisum sativum</i>				
Canner's Champion	12	0	24	0
Gradus	12	0	24	0
<i>Capsicum annuum</i>	5	0	5	0
<i>Solanum melongena</i>	5	0	2	0
<i>Raphanus sativus</i>	5	0	5	0
<i>Viola tricolor</i> (pansy)	5	0	5	0
<i>Aster</i> sp. (aster)	5	0	5	0
<i>Spinacia oleracea</i>	1	0	2	0
<i>Lactuca sativa</i>	5	0	10	0
<i>Althaea rosea</i>	1	0	2	0
<i>Malva rotundifolia</i>	2	0	5	0
<i>Lamium amplexicaule</i>	1	0	3	0
<i>Amaranthus retroflexus</i>	2	0	4	0

the plant. From general observations it appears that the stunting and dwarfing are progressive from season to season.

Because of this characteristic stunting and decline of delphinium affected with this virus, an appropriate name for the disease is "Stunt," which is here proposed. Infected delphinium plants grown in the open were slower in starting growth in the spring than healthy plants. Some plants showed extreme dwarfing with a chlorotic appearance. The foliage of these plants was extremely dwarfed and mottled or chlorotic with savoying and cupping of the laminae in varying degrees (Fig. 2, B).



FIG. 1. A. Healthy delphinium. B. Dwarfing and floral proliferation due to natural infection of the virus.

The chlorosis was confined to the margins of the leaves. In addition, dark brown to tarry black necrotic specks and streaks of variable sizes and shapes up to 5 mm. or more were often evident on the leaves (Fig. 2, C). When these brown, necrotic, dead areas were confined to a single marginal spot the leaf became distorted due to the uneven marginal growth (Fig. 2, C). In many cases these necrotic areas would coalesce and involve entire leaves and thereby produce complete killing. Leaf petioles and stems often exhibited similar necrotic spots and streaks. It was not uncommon to find tarry black lesions 2 to 3 inches in length completely girdling the stems and petioles and thereby causing death of the affected shoots. New shoots arising from such devitalized plants were more numerous and materially stunted. All of the above symptoms were evident on the first spring growth and continued until about the first of June. Severely infected plants on that date were only 6 inches tall in contrast to 2 feet or more in height for

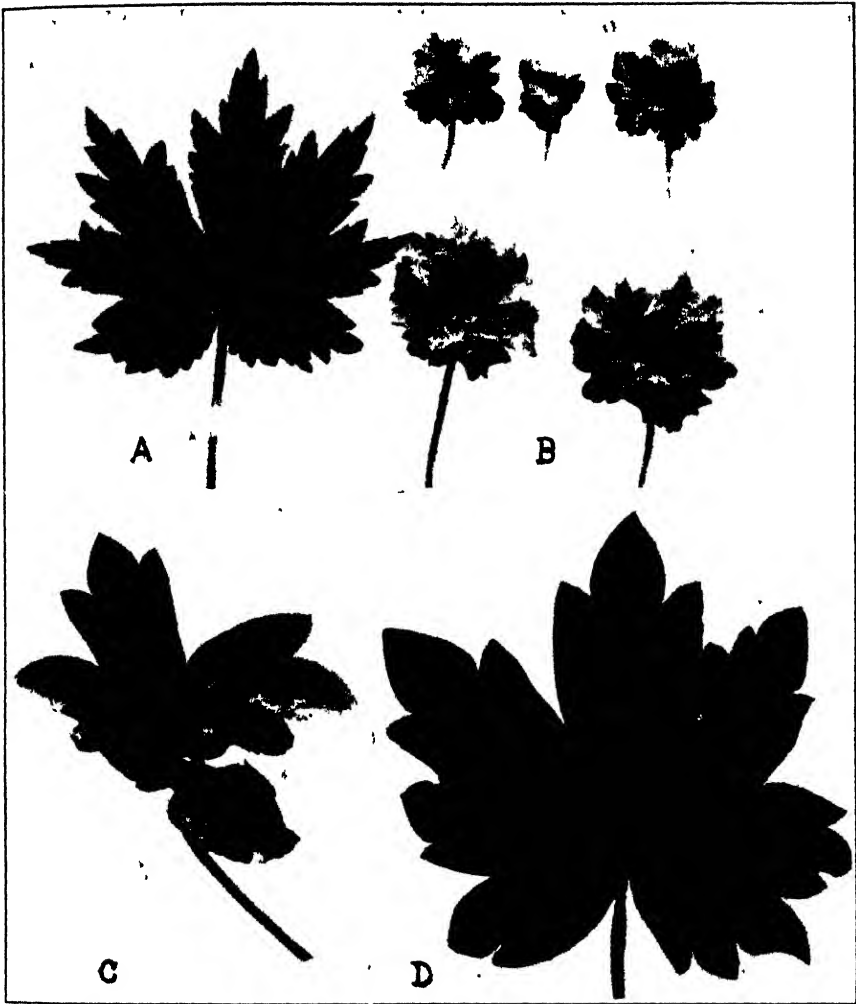


FIG. 2. A. Normal leaf of delphinium. B. Foliage with dwarfing, mottle, chlorosis, savoying, and cupping of the laminae. Field occurrence. C. Tarry black necrosis of the leaf, natural infection. D. Tarry black ring and line pattern of the leaf as a result of artificial inoculation in the greenhouse.

healthy plants or those exhibiting slight symptoms. Later in the summer there was an excessive proliferation of the flowering stalks, which produced a bunchy witches' broom appearance (Fig. 1, B). The flowering parts failed to develop normally but produced a characteristic leafy proliferation with varying degrees of transformation from slightly greenish flowers to pale green leafy structures (Fig. 3). Some of the severely infected plants in a plot grown by the horticultural department died early

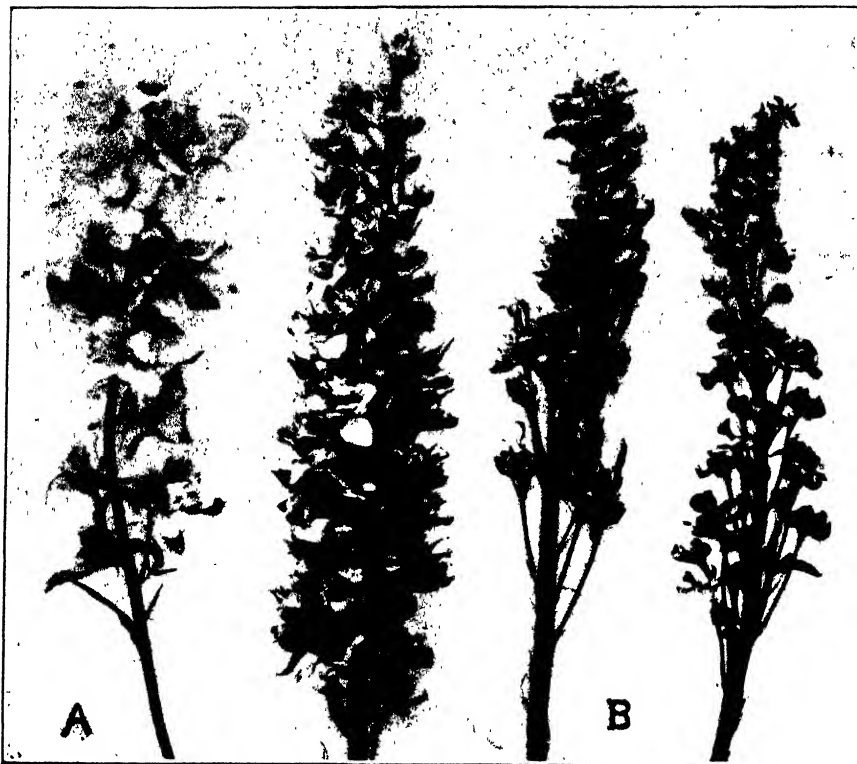


FIG. 3. A. Healthy inflorescence. B. Leafy proliferation of varying degrees of the floral parts. Field occurrence.

in the fall, following the production of the aborted floral parts. When such plants were examined it was found that the roots had so decayed and disintegrated that the stalks could be easily pulled or lifted out of the soil. The symptoms as described above were evident on plants grown under normal conditions. Because seedlings of delphinium, inoculated with the virus (recovered from delphinium) have not yet matured in the open, it cannot be stated definitely that the transformation of the floral parts to leafy structures or the premature dying of the plants in the fall is definitely associated with the virus. Inoculated delphinium seedlings grown in the greenhouse have exhibited the other symptoms that are common to plants grown in the open.

The virus, which has been recovered on tobacco, when transferred to six-months-old delphinium seedlings, during October, produced a slight yellowing of the foliage and a dark brown necrotic ring and line pattern (ring spot type) on the inoculated leaves (Fig. 2, D). Streaks or blotches of brownish black necrotic areas soon appeared on the petioles. The following

spring the characteristic tarry black necrotic spots appeared on the new foliage.

Plants exhibiting this disease have been reported from various sections of the State. One nurseryman stated that it was quite common in his plantings and that he had received complaints from various customers in different parts of the United States that some plants received from him failed to produce satisfactory growth. From this and other available information it would appear that the disease is quite generally distributed throughout the United States. There is some evidence that it has been present in this State for at least 15 years.

#### SYMPTOMS ON TOBACCO

Inoculations were made to *Nicotiana tabacum* (Connecticut Havana) plants in October, 1932, from affected delphinium that had been transplanted into boxes in the greenhouse. The delphiniums produced sufficient foliage from which to secure inoculum. With the first transfer from delphinium to tobacco, symptoms may not appear until about the 15th to 20th day, but subinoculations to tobacco more often produced symptoms in 4 to 6 days. The symptoms on tobacco are variable, sometimes showing as a white, necrotic etching, with ring-and-line pattern of variable forms, while in other cases the first recognizable symptom is a vein clearing (Fig. 4, B). Some plants never produce the ring-and-line pattern but exhibit a vein clearing that later changes to a blotchy, irregular, usually interveinal mottle which may coalesce to form extensive blotchy chlorotic areas (Fig. 4, A). In more mature plants these blotches may involve the major part of the older leaves. In addition the tobacco leaves usually are somewhat ruffled, producing a wavy and crinkled appearance. Some leaves may appear normal but subsequently produce a faint mottle which may or may not develop into the blotchy type. The necrotic ring and line patterns are confined to the first few leaves, while the older plants seldom show evidence of this symptom but rather exhibit the blotchy type of foliage. In most plants inoculated with this virus, dwarfing is usually very evident.

This virus, when combined with tobacco mosaic, appears to intensify the symptom expression of the tobacco mosaic on tobacco. The combined viruses sometimes produce symptoms comparable to white mosaic of tobacco, and also, sometimes, cause some vein or leaf necrosis. The delphinium virus, when combined with the latent virus of potato, generally produced a type of spot necrosis similar to that produced by the combination of veinbanding and the latent virus. In a similar manner the delphinium virus and veinbanding produced a type of spot necrosis similar to the above combination. From this it appears that the delphinium virus is variable

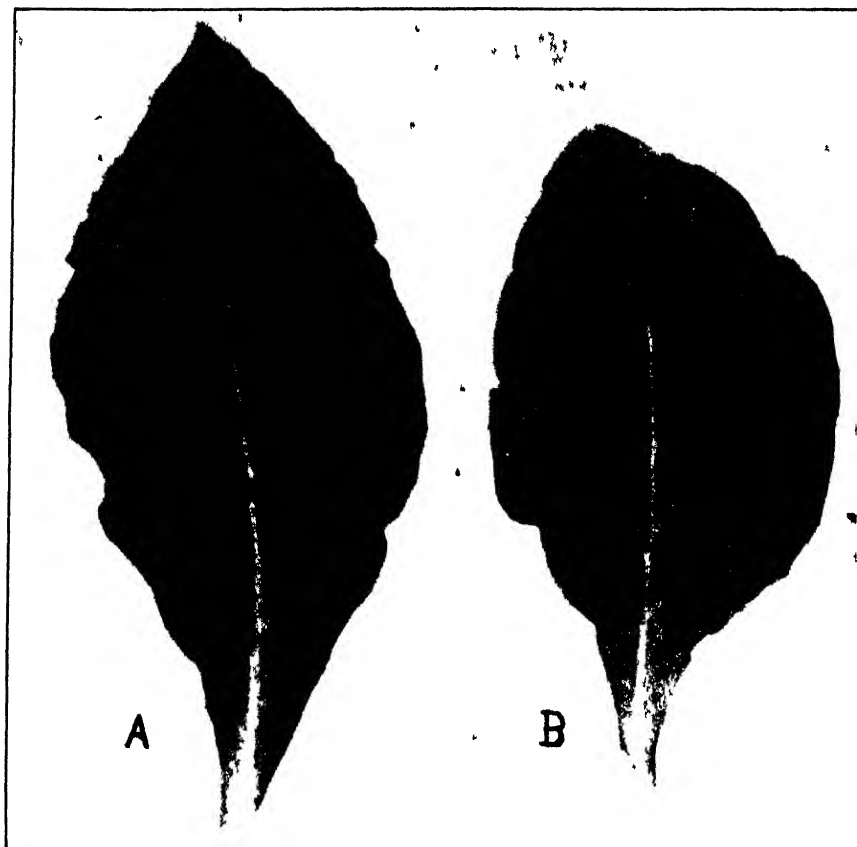


FIG. 4. Havana Connecticut tobacco inoculated with the delphinium virus. A Blotchy, irregular mottle. B White necrotic etching with ring and line pattern.

and capable of producing variable symptoms when combined with other specific viruses.

#### SYMPTOMS ON TOMATO

The delphinium virus is readily transferred to tomato, producing characteristic symptoms. The plants at the end of 12 to 15 days usually are dwarfed when compared with healthy checks. The first symptom, other than dwarfing, is a definite vein clearing, evident between 15 and 20 days after inoculation. This is followed within a few days by a definite downward rolling of the leaflet margins, a hooking down of the ends of the leaflets as well as the apical part of the leaf and the leaflets often become deeply lobed or cut (Fig. 5). The foliage at this stage shows a faint but perceptible mottle, which intensifies with the age of the plant. The interveinal tissue becomes lighter colored in contrast to the





FIG. 5—John Baer tomato exhibiting deeply lobed leaves and curling and distortion of leaves and stems as a result of inoculation with the delphinium virus

darker areas bordering the veins. As this mottling increases there appear irregular large blotchy areas on the leaves similar to those described on tobacco. This blotchy type of mottling is quite characteristic also on several of the susceptible hosts reported below. Plants infected with this virus usually exhibit a pale green color, and there is a slight to moderate savoying of the leaves. The fruit, however, evidently is not affected. These symptoms are soon followed by further distortion of the leaves in which they become curled in a corkscrew fashion, turning on their axis as much as  $180^{\circ}$  or more. This curling also occurs on the main stem or on lateral branches (Fig. 5).

*Combination on tomato.* The delphinium virus in combination with tobacco mosaic produced on the tomato extreme and variable symptoms. In addition to the symptoms resulting from either virus alone there appeared definite filiform leaves (Fig. 6) and some plants exhibited a type of streak (Fig. 7), somewhat resembling those produced by the latent virus and tobacco mosaic. The former combination caused dwarfing, necrosis of the leaves, and some petiole streak, but there was less apical killing, stem and petiole streak, or the greasy brown lesions on the fruit that is common with the latter combination.



FIG. 6. Filiform leaf of tomato produced by the combination of the delphinium virus and tobacco mosaic.

The delphinium virus, when combined with the latent virus of potato, gave, in addition to the symptoms produced by either virus alone, a type of spot necrosis and some killing of the lower foliage. It caused also a type of mottle closely resembling mild symptoms of tobacco mosaic.

The combination of the delphinium virus and veinbanding failed to give additional symptoms to those produced by these viruses when found alone on tomato.

#### SYMPTOMS ON OTHER HOSTS

*Apple of Peru, Nicandra physalodes.* The first evident symptom appearing in 4 or 5 days consists of water-soaked areas of variable size.



FIG. 7. A type of streak of tomato produced by the combination of the delphinium virus and tobacco mosaic.

These soon coalesce, resulting in large irregular brown necrotic lesions that may involve the entire leaf and cause it to die and fall from the plant (Fig. 8, C) with the possible resultant death of young plants. In any event the plant is materially dwarfed; but, if not killed, it will make a slow

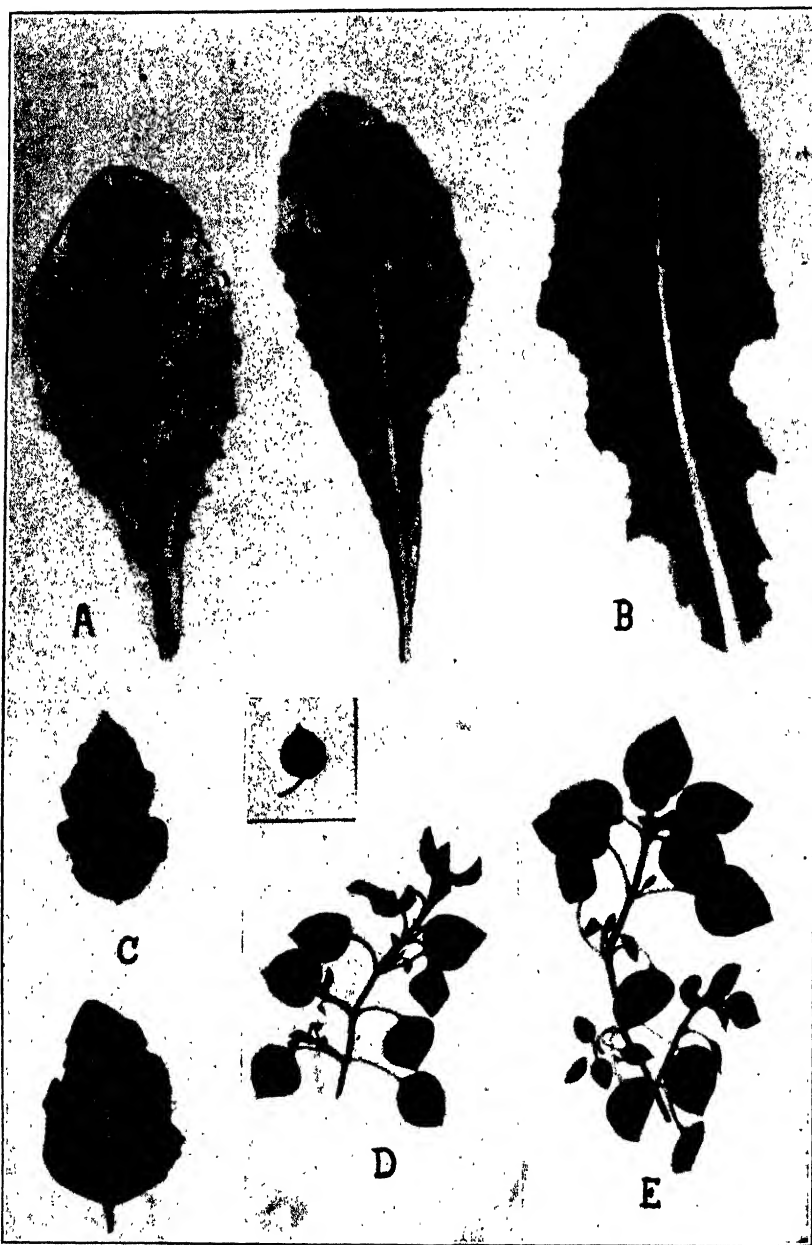


FIG. 8. A. Dwarfing and blotchy mottle on *Lactuca scariola* produced by artificial inoculation with the delphinium virus. B. Normal leaf of *L. scariola*. C. Brown necrotic lesions produced on *Nicandra physalodes* by the delphinium virus. D. Mottle and folding of leaves of *Stellaria media* produced by the delphinium virus. E. Normal stem and leaves of *Stellaria media*.

growth. The new foliage often may fail to produce evident symptoms; but the virus is systemic, since it can be recovered from foliage that fails to exhibit symptoms.

*Black Nightshade, Solanum nigrum.* The symptoms produced on black nightshade when inoculated with the delphinium virus closely resemble those on this species when inoculated with tobacco mosaic. The most common symptom is a mottling of light and dark green areas. There is no appreciable savoying of the foliage with either tobacco mosaic or delphinium virus, but with the latter there is a more conspicuous irregular blotchy type of mottle, which corresponds to the blotchy mottling on tomato and tobacco plants. This host appeared to be less susceptible than tobacco or tomato.

*Chickweed, Stellaria media.* When chickweed was inoculated with the delphinium virus it became dwarfed and produced a mottle with chlorotic interveinal tissue. The veins usually retained their green color but, in some cases, they also became chlorotic (Fig. 8, D). The apical leaves usually became completely folded over, with the midrib as the central point of the fold (Fig. 8, D).

*Prickly Lettuce, Lactuca scariola.* The symptoms on prickly lettuce consist of dwarfing, a definite curling and folding of the leaf with a blotchy mottle which is produced by numerous small, white, necrotic areas (Fig. 8, A).

*Cucumber, Cucumis sativus.* The more pronounced symptoms on cucumber foliage consist of a mosaic mottle and rigidity of the foliage similar to that produced by true cucumber mosaic. Often, there is also a sharp downward or an upward bending of the distal portion of the leaf. This turning downward or upward may be slight or it may form an angle of 90°. Some fruit which matured failed to show evident symptoms.

*Zinnia, Z. elegans.* The more evident symptoms on zinnia consist of dwarfing, excessive crinkling, and rigidity of the foliage. There was no apparent mottle evident.

*Horehound, Marrubium vulgare.* The only evident symptom on horehound is a general dwarfing.

*Other Susceptible Species of Plants.* Symptoms were not evident on dog fennel, *Anthemis cotula*, shepherd's purse, *Capsella bursa-pastoris*, or petunia, *Petunia hybridum*, when inoculated with the delphinium virus, but the virus was recovered from each when subinoculations were made to tobacco plants (Table 2). In each case a young leaf, above the point of inoculation, was taken as a source of inoculum from these plants about 3 weeks following the original inoculation.

*Nonsusceptible Species of Plants.* Several unsuccessful attempts were made to recover from the following 16 plants the delphinium virus with

which they were inoculated: *Solanum tuberosum* (virus-free Early Rose and seedling stock), *Datura stramonium*, *Solanum pseudocapsicum*, *Chenopodium album*, *Pisum sativum* (Canner's Champion and Gradus varieties), *Capsicum annuum* (pepper), *Solanum melongena* (egg-plant), *Raphanus sativus* (radish), *Viola tricolor* (pansy), *Aster* sp. (aster), *Spinacia oleracea* (spinach), *Lactuca sativa* (lettuce), *Althaea rosea* (hollyhock), *Malva rotundifolia* (mallow), *Lamium amplexicaule*, and *Amaranthus retroflexus*.

#### DISCUSSION

It has been shown by other investigators that delphiniums in a few sections of the United States are affected with a virosis and that other diseases not definitely proved are presumed to be of virus origin. The virus that has been used in this study is capable of producing extremely variable symptoms on delphinium and some other plants. For this reason it is not at all improbable that some or all of the symptoms may be expressed under different environmental conditions. From this point of view it may be assumed that a single virus is responsible for these variable symptom expressions. Delphinium, inoculated with this virus, produced such symptoms as ring spot, necrosis, and chlorosis similar to those reported by other authors. Plants from which the virus was secured exhibited such symptoms as necrosis, chlorosis or yellowing, a witches'-broom effect, and a reversion of the floral parts to leafy structures.

The delphinium virus appears to be capable of causing a different response when combined with other viruses. When combined with the latent virus of potato, it produced a type of spot necrosis similar to that resulting from the combination of the veinbanding virus and the latent virus. Similarly, it caused a type of spot necrosis on tobacco when combined with the veinbanding virus. It is evident that the delphinium virus can be substituted for either the veinbanding or the latent virus to produce spot necrosis on tobacco plants. Again, when combined with tobacco mosaic, it responds much the same as the latent virus when in combination with tobacco mosaic. This virus, plus tobacco mosaic, caused a type of streak on tomato and leaf necrosis of tobacco similar to those produced by a combination of tobacco mosaic and the latent virus. Again, it is capable of producing fern leaf of tomato when combined with tobacco mosaic and therein resembles the reaction of cucumber mosaic on tomato. The fern leaf is not caused by the delphinium virus, alone. It would then appear that this virus has two rather peculiar properties in that it is capable of producing such variable symptoms on many different species of plants and that it is variable in its response when combined with other viruses.

## SUMMARY

The virosis of perennial delphinium known as "stunt" or witches' broom is common in Washington, Idaho, and other portions of the United States and causes a dwarfing, an increase in the number of shoots, various foliage symptoms, including dwarfing, mottling, chlorosis, savoying or cupping of the laminae, necrotic patterns on both stems and leaves, and greening and malformation of floral parts.

The virus has been transmitted by mechanical methods to tobacco, tomato, cucumber, apple of Peru, black nightshade, chickweed, prickly lettuce, zinnia, horehound, dog fennel, shepherd's purse, and petunia, causing visible responses of various types on all except the last 3, on which it appeared to be latent, as determined by reinoculations into tobacco.

The virus caused no visible effects and also was not latent on 16 other species of cultivated and wild plants, as determined by inoculations on tobacco. No other susceptibles have been known to contract the disease under natural garden conditions.

The delphinium virus, when combined with tobacco mosaic, intensifies the symptom expression on tobacco, causes variable symptoms on tomato, including string-like or filiform leaves and a type of streak; when combined with the potato latent virus on tomato, symptoms not shown by either virus alone were exhibited.

The vectors of the delphinium virus have not yet been determined.

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# VITALITY AND VITALITY DETERMINATION IN POTATOES<sup>1</sup>

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When, at the beginning of this century, the diseases of potatoes became increasingly more frequent, with the resultant unfavorable effect on the crops, the agriculturists interested in potato culture became somewhat disturbed. Everywhere the leaf roll, curl, and mosaic and other causes of damage became particularly prevalent, in part, in disquieting proportions.

Since the scientific investigations into these various diseases—collectively called degenerative diseases—failed to bring quick results, and in as much as their eradication became an urgent necessity, ways and means of evaluating the seed potatoes were adopted; in other words, the state of health of the crops from which seedlings were to be taken was determined. This method of evaluation has spread from Germany all over the world. I myself was able to cooperate in 1914 in the development of appropriate measures in the United States.

In the meantime, however, it had become apparent that, even with tested seedlings, there was no absolute certainty of healthy crops. On the contrary, it can be noted more frequently that potatoes from an apparently healthy field produce defective crops the following year. This has led us to investigate the possibility of determining from the seed potato itself whether or not it will produce a healthy plant. Thus we came to formulate the concept of tuber vitality.

Conditions are more or less similar with grain. At first, one was guided by the determination of its germinability; but later, when one could see that this was not sufficient, one proceeded to the determination of the germinating power. One has determined, therefore, not only the presence of vitality but also the degree of vitality. A similar procedure was not possible for potatoes, since the germinating conditions of the potato do not indicate the state of its health. We, therefore, had to approach the subject from a different angle and examine the various conditions of chemical composition and of vital manifestations. We have succeeded to arrive, by two different routes, at useful results. I am particularly happy to be able to present these results to you today.

It is not my intention to discuss the various degenerative manifestations themselves, nor to detail the various theories and experimental results related to them. I shall rather report to you on the copper method of Bechhold and Erbe and the potentiometric determination of the seed quality

<sup>1</sup> Paper read by Dr. Appel at the meeting of The Am. Phytopath. Soc.—Chicago “Century of Progress” program, June 21, 1933.



according to Wartenberg and Hey. The former is the result of cooperation between the Biological Reichs Institute for Agriculture in Forestry in Berlin-Dahlem and the Institute for Colloidal Research in Frankfort a. Main, the latter originated in the Botanical Laboratory of the Biological Reichs Institute.

#### THE COPPER METHOD OF BECHHOLD AND ERBE

The copper method appears at first to be very simple. However, in spite of its simplicity it contains several very important sources of error. Therefore, it will be necessary for me to call attention also to these errors.

The preparation of the tubers for examination is quite simple. Take a strip of copper, 1.5 cm. wide and 10 cm. long and insert it lengthwise into the tuber so that a piece of copper projects from either end. The tuber thus prepared is then placed in a warm moist chamber, which is placed in a thermostat. The tuber remains here for 8 hours at 38° C. Then the moist chamber is taken out of the thermostat and is allowed to stand for 16 hours in a room at room temperature, *i.e.*, at 18 to 20° C. Thereupon the copper strip is removed from the potato and the tuber is cut in two. The cut should be made in the direction of the insertion but at right angles to the flat surface of the copper. On the cut surface, along the channel made by the copper plate, there appears a more or less intensely colored zone that, at first, is brownish or reddish but assumes, upon exposure to the air, a dark gray or black tone. The extent of the discoloration is characteristic for the degree of health or degeneration of the tuber. A degenerated tuber shows none or only a trace of discoloration, while a healthy tuber shows, in typical cases, a broad zone of color, which is of a dark tint around the point of insertion.

However simple the operation of this method might appear at first glance, the evaluation of the results is difficult: because, as mentioned above, there exists a series of sources of error that are difficult to control and that may influence the results. First of all attention must be paid to the exact maintenance of the temperature of 38° C. in the thermostat. This temperature must be positively obtained and maintained throughout, otherwise comparative evaluations of cropping values are impossible with this method. The principal difficulty lies in the accurate evaluation of the discolored zones; not so much because such an evaluation is difficult because of the different picture presented by each cut surface as a result of the irregular outline of the edge of the zone, but rather because it is certain, even today, that the extent of the discolored zones in some cases is affected not only by the degree of degeneration, but also by the variety and other specific considerations of the origin of the seed. Thus, for instance, there are varieties whose discoloration in cases of vitality are smaller or larger than that of

other varieties. It also happens that, within one and the same variety, seed from one origin shows no discoloration—because of influences yet unknown—and thus presents the picture of a degenerated potato, while later on in cultivation a perfectly vital growth is obtained. The reverse case, where a degenerated tuber presents the picture of a healthy one, is very rare and has not yet been positively demonstrated.

At present investigations are being undertaken for the purpose of eliminating these sources of error in order to make the method practical for the farmers. Unfortunately, the specific method of examination and the impossibility of looking at the interior of the tuber and the changes that are taking place within it, make it difficult to recognize the sources of error. It is hoped, however, that in the near future the copper method of Bechhold will become a reliable method for the practical agriculturist.

Nothing can yet be said concerning the details of the copper method. It is the result of accidental observation and we do not yet know today why discoloration occurs in a healthy tuber and why the inside of a diseased tuber does not discolor. Just as little do we know about the rôle played in the whole process by the copper strip. All that has thus far been found in this respect must be designated as uncertain experimental results. These, however, already show that we are confronted with the most difficult problems of physiology. The copper method, as well as another that I shall explain to you later, are the practical achievements of scientific experiments that may be put to practical use within a reasonably short time from now. Furthermore, they also point out for us the way to a new type of experimentation that will perhaps bring us a great deal nearer to the solution of the problems of potato degeneration.

#### WARTENBERG AND HEY'S POTENTIOMETRIC METHOD OF EVALUATION OF CROPPING VALUES

Every practical method requires mastery over sources of error. In this respect Bechhold's copper method is outdistanced by far by the potentiometric method of evaluation of cropping values elaborated in the Botanical Laboratory of the Biological Reichs Institute by Wartenberg and Hey. This method can boast today of extensive investigation and a considerable series of successful evaluations for practical purposes.

The preparation and operation of the measurements are not so simple, and a great deal more experience is required for the evaluation of the individual tests than is necessary with the copper method. On the other hand, however, the results obtained by Wartenberg and Hey's method have proved so far to be considerably more reliable and give, with a sufficient number of individual determinations, not only a definite average value for the degree of degeneration of a certain variety but also an irreproachable

picture of the percentage distribution of healthy and diseased tubers within one and the same variety.

The seed potatoes that are to be examined as to their cropping value are each separately washed, peeled, and grated to a mash on a glass grater. The mash of each tuber is put into a beaker and mixed with double-distilled water in the proportion of about 1 to 2. After stirring, the froth is removed from the mash by means of a glass spoon. A clean platinum electrode is then inserted into the center of the settled mash in each glass. The platinum electrode should first be cleaned with sulphuric acid and well rinsed with tap water and distilled water. The above arrangement represents a half element, since the platinum of the electrode receives an electrical charge in the mash, and in the course of time there develops on the platinum sheet a definite electric potential.

Such a half element may be compared to  $\frac{1}{2}$  of a pocket battery. Therefore, if it is being connected with another half element, converting it into a complete element or a so-called double half element, it is possible to obtain an electric current from this arrangement. If a second half element, one with a known constant potential, is taken, it is then possible to measure the difference of potential of the double-half element and to calculate the electric charge which the platinum sheet has received in the potato mash.

In the potentiometric method of evaluating cropping value according to Wartenberg and Hey, such a double half-element arrangement is employed and the difference of potential of the two element poles measured. As a parallel half element for purposes of comparison, the saturated calomel electrode, which always has a constant potential, is employed. It is connected on one side by a KCl-electrolyte bridge with the mash of the potato half element, and on the other side the current between the 2 metallic poles is connected by means of a wire connection with the measuring apparatus. As measuring apparatus serves the Trenel Potentiometer by means of which the difference of potential of the 2 half elements is measured according to Poggendorf's Compensation Method. We have constructed recently in Dahlem a measuring apparatus with which one person is able to measure 40 tests simultaneously.

The time element in the determination of each test can be divided into 2 parts. The first part comprises the duration of the initial curve, that is, the time in which the potential of the electrode adjusts itself to the constant value; and the second part serves as a control of the constant value. The value obtained at the beginning of every individual measurement is arbitrary and has no relation to the degree of degeneration of the potato tuber, which has been utilized to make the potato half element. The latter, at the very beginning of the test, may represent either the positive or the negative pole of the double half element. If, however, the readings are repeated in

intervals of about 10 minutes, it will be noted that during the readings the difference of potential between the potato half element and the control electrode—the calomel electrode—changes. The potato half element in the course of time becomes more and more negative in relation to the calomel electrode. Finally, the change decreases and then the difference of potential remains constant. This is the end value towards which the initial curve is aiming. The end value is positively ascertained by means of 2 to 3 hours of control of this value. It must not change any more during this period.

As mentioned above, the initial reading of a test bears no relation to the degree of degeneration of the tuber. This initial value together with the entire first part of the readings represents a necessary evil in this method that it has yet been impossible to circumvent.

The question may be asked why the end value is reached only after a curve has been established, sometimes over several hours. We have studied this question in the course of special investigations because it is of the greatest importance for the accuracy of the method. These investigations have not yet been completed, but they have already shown that the results are highly interesting from a theoretical viewpoint and that they satisfy the practical requirements; in other words, the theoretical basis of this method is firmly established.

With regard to the practical operation of the method, it may be stated that the end value depends upon the beginning and the general course of the initial curve. The 2 latter points are determined by the preparation of the electrode, the sensitivity of the measuring apparatus, the specific characteristics of the potato tuber, which, however, do not affect the end value, and most of all by the indefinable ability of the person who is using the measuring apparatus.

A description of the experiments, however interesting they might be theoretically, would be going too far. I have the task to report on practical matters and shall, therefore, limit myself to a description of those measures that have a practical significance for this method.

One of the reasons for the initial curve is the original charge of the electrode, which depends upon the character of the cleaning material as well as upon the length of time during which the electrode has been in contact with the air. In our own case, the original charge is normally always positive. The change in the charge of the electrode in the potato mash does not occur suddenly, but requires a certain time expressed in the gradual change of the measured difference of potential.

Another reason for the initial curve is the polarization of the electrode. This is explained by the fact that, at the beginning of the test, one does not know in which range of potential the end value will lie. Therefore, one

establishes arbitrarily a compensative resistance and thereby, if it is too small, sends an electric current to the electrode that is then electrically charged. The effect of polarization is similar to that of the original charge; some time is then required until the action of the potato mash on the charge of the electrode becomes manifest.

A third reason for the initial curve is to be sought in the potato mash itself. The electric charge, which has been communicated to the platinum electrode by the potato mash, is tied up in the latter with a substance that is unevenly distributed in the tissue suspension shortly after its preparation. There must be first a neutralization of concentration so that this substance becomes present in the fluid of the mash in the same concentration as in the suspended tissue particles. Until this has happened, the constant potential cannot be attained. The neutralization of concentration, the diffusion of a substance from the intact cells of the tissue particles into the diluent, is a process that requires a certain time. The establishment of the potential is a definite function of time, simply because the substance that diffuses from the cells influences the potential, or rather the formation of the potential in a definite direction. It is definitely established only when a difference of diffusion of the substance from the cell to the surrounding fluid no longer exists.

The first and the third of the above described reasons for the initial curve may be methodically eliminated merely by postponing the readings until the following day. Immediately after the preparation of the potato half-element, the mash together with the electrode in it, are left overnight in an ice box at a temperature of about 10° C. The initial charge of the electrode is no longer present in the morning, and the neutralization of concentration is then complete. Then, under these circumstances, one still obtains an initial curve which, however, depends solely upon the intensity of the effect of polarization. If the readings are begun after establishment of the compensative resistances, which lie near the range of potential of the expected constant, polarization is unimportant and the aim of the measurement is soon reached. In this manner we have reduced the duration of a test from 10 to 4 hours, in which are included the 2 to 3 hours of control of the constant potential. I am anxious to emphasize once more that the half-elements must be positively kept in an ice box at the required temperature until they are used for the test. Higher temperatures might, it is true, hasten the neutralization of concentration, but they would have the drawback of hastening the fermentation of the tissue mash. If fermentation occurs, then the cropping value can no longer be determined. Through fermentation, one obtains potentials that lie in a different range from that of the potentials of healthy cropping values. Furthermore, the formation of potential through fermentation is characterized by the fact

that it does not lead to a constant; it gives, from reading to reading, progressively negative results.

On the other hand, it is not necessary to preserve the potato half-elements in the ice box at temperatures lower than  $10^{\circ}$  C. All that would be achieved would be to retard the neutralization of concentration.

The aim of every measurement is, as we have said above, only the constant value. If it has been obtained under proper conditions and if it has been controlled long enough, if it can be looked upon as definite, then the test may be terminated. The beginner should be certain to continue the control of the constant value for at least three hours. The constant value of the potential of the potato mash is in direct relation to the degree of degeneration of the potato. If it lies within a range of from  $-100$  to  $-140$  millivolts in relation to the calomel electrode, then we have before us, at any season and for any variety, healthy tubers, only, that will produce healthy plants in every case. Constant values that are more than  $-210$  millivolts in relation to the calomel electrode always represent diseased plants in all cases. Those tests that become constant between  $140$  and  $210$  millivolts, however, should be evaluated according to the variety and the season in which the measurements have been made. I shall report in more detail later on the influences of variety and season.

First of all, I would like to demonstrate to you how Wartenberg and Hey have proved the relations between the measured values of constants and the degree of degeneration of the tubers. They sectioned the potato tubers by a cut from the crown to the stem, prepared one of the halves for measurements, and determined its value of constant, while they planted the other half in a greenhouse or in the experimental field where the growth of the plant from the tuber halves could be observed and compared with the measured cropping values. At present several thousands of such comparisons are available and have given good results. If the tubers of one or of different sources of seed potatoes of one or several varieties are planted in this manner in the field or in pots in sufficient numbers, according to their measured values, we note that, for example, with the variety "Erstling," which should be measured in January, healthy plants grow from the tuber halves of the first value range approximately up to the value of calomel electrode minus  $195$  millivolts. Then there follows another group within the next  $15$  to  $20$  millivolts approximately up to value  $210$ , in which occur overlaps and in which the relations between potential values and degree of degeneration are not pronounced, where, therefore, healthy and diseased plants may fall within the same range. We speak here of a so-called *critical zone*. Below this critical zone, that is to say at values higher than  $-210$  millivolts in relation to the calomel electrode, all plants are diseased. Up to the present it has not been possible to determine the type of disease

by means of the measured potential values. The relation between tuber potential and degree of degeneration, therefore, is not indicated in that, within a definite range of the constant values, only the leaf-roll disease is present, or in another range only the leaf-curl or mosaic is present, but merely indicates the difference between healthy and diseased tubers. The degenerative symptoms of leaf roll, curl, mosaic, bucket, dwarf, etc., appear to be primarily related to the variety and also to the source and origin of the seed. If the mean value of a certain source of seed lies lower in the disease range of the potential scale than that of another, the degree of degeneration is detectable also higher, that is, the entire vegetative potency is less under the same conditions of growth.

The outstanding advantage of the method I have just described to you lies in the fact that by means of this determination of vitality, all subjective errors can be excluded. On the other hand, the method has the drawback that the application of the test must be learned. The person undertaking such investigations must not only know the manner in which the test is managed, but must also be well acquainted with the errors that may arise. Furthermore, it is important to know how the values are to be judged during the different seasons and how one should deduce from a series of measurements a definite idea of the degree of the cropping value of tubers from a certain source.

In every investigation it happens that one or more measurements must be discarded because of possible errors. The fact that errors are easily recognized is another advantage of the method. The failures just mentioned may occur in different ways. The sources of error that may influence the individual measurements are divided into 2 classes. First, they may be errors having their origin in individualities of the measured tuber. The second group comprises errors that are traceable to outside influences. In all cases, the error manifests itself in an abnormal run of the curve, when the constant is either not attained at all, or else the curve ends in an apparently constant value, the position of which in the scale of potentials, however, is so improbably high or low that it falls outside the range of variation because of origin, and in this manner the error becomes visible at once.

The electrode failures are outstanding in the group of failures influenced by outside factors. New electrodes cannot be used for accurate measurements for a number of days. At times their potential differences in the same mash differ considerably from those of standard electrodes that have been in use for a long time. After the new electrodes have been used 8 or 10 times and have passed through a cleaning process, they give the correct value. One must therefore clean and test new electrodes until all of them

give nearly the same values in the same mash. The greatest permissible variation is 4 to 5 millivolts between electrodes with extreme values.

In order to make clear one failure against which one should be very much on guard, I shall now describe to you the type and form of the electrode. On a sheet of platinum, one square inch in size, is soldered a platinum wire, which passes through a glass tube and is connected at its upper end with a contact. The platinum sheet is fused partly into the lower end of the glass tube so that the upper fifth of its surface is covered with glass. The ordinary glass cannot be fused tightly enough on metallic platinum. Therefore, it is necessary to fix a piece of lead glass in the lower end of the glass tube into which the platinum sheet can be fused. In spite of these special measures, it may happen that cracks arise in the joined area, which often cannot be detected with the naked eye. The effect of such cracks manifests itself in potentials that are strongly displaced towards the positive side. This is due to the fact that the cleaning material enters the cracks during the cleaning process; it cannot be washed out and, later on, affects the formation of potentials. The influence is so great that it is noticeable in the course of the measurements. It produces, then, values that fall outside the normal range.

The position of the platinum sheet in the test beaker is of the greatest importance for exact measurements. One must make sure that it is inserted into the suspended tissue mash. It should not extend into the upper clear solution nor into the settled starchy layer at the bottom. If it does, the result would be a delayed curve, which, under certain conditions, does not become constant, or else the slow setting of the electrode may mislead the inexperienced observer into believing that a constant value has been obtained.

Fermentation, because of its causes, really belongs in the second group of errors, which I shall describe later. It manifests itself in a rapid fall of the potential that may reach from 300 to 400 millivolts below the lower border of the range of the potato potential. In most cases, the falling off on account of fermentation occurs only after hours of normal constancy. Certain tubers may show fermentation curves sooner than others. The reason lies in a yet unknown property of the tuber. In most cases, however, fermentation may be retarded and thus a sufficiently long constancy obtained by shortening the duration of the test by means of the above mentioned storage in an ice box.

More difficult than the recognition and elimination of the already described failures is, in many cases, the evaluation of those that arise from certain properties of the tubers. Particular attention is required with regard to the formation of melanine, the black discoloration, well-known to any one who has ever handled potato mash. The normal case in which



measurements may be made without difficulty is present when, before the beginning of the test, there is a clear distribution in the solution standing above the settled uncolored tissue. A zone of clear uncolored solution is present above the mashed tissue and a dark zone is sharply defined above this layer. This latter zone is dark brown to black, colored by colloidal melanine. The black zone of melanine formation does not occur in many varieties of seeds of different sources but remains a light to dark red. Since, also, there is here present a zone of colorless liquid between the mash and the layer of melanine, measurements are possible. Whenever the red color extends into the mash and, therefore, to the electrode, the test must be discarded. Likewise, all those tests must be discarded that show no or only a trace of melanine formation because, in normal cases, the melanine acts as a buffer against the oxygen of the air. If this oxygen, which penetrates into the sample during the preparation of the mash, were not eliminated by the formation of melanine, measurements would not be possible at all.

Tubers that show rust spots must be excluded from the very beginning. They cannot be measured. Their potential deviates toward the positive side and becomes constant in a range that does not correspond to their true cropping value. The same suspicion of an abnormal constant value should be applied to tubers infected with bacteria or fungi. Small visible infections can be cut out of the tuber. The formation of potential in the remaining part of the tuber is not influenced by this procedure. The greatest precautions must be observed in cases in which the infection is difficult to determine, as in the case of black ring. We know but little about these matters, but I believe that a number of failures must be debited to them, since we have as yet no other explanation.

Abnormal incidents in the measurements of tubers may appear for all varieties in autumn, that is, before winter dormancy. The curves of the measurements at first fall normally, then rise irregularly, and eventually fall again. In such measurements it is not possible to obtain constant values. This phenomenon is one of the most exceptional processes during the actual winter rest of the tuber. It may be observed again with a few, but not with all, varieties in the spring, when the winter dormancy is terminated.

All these errors that manifest themselves in the rising of the curves and in the lack of constant values are very rare. If one compares these cases in relation to their number with normal measurements, they appear almost negligible. I would like to emphasize this fact particularly because, in enumerating the great number of possible errors, you might be led to believe that the method is uncertain. Quite the contrary; because, by recognizing all sources of error, we have been able to guarantee this method to an extent that it is useful and valuable if applied by skilled workers.

Now, this still leaves the question as to the best time at which the measurements should be made and what part the properties of the different varieties may play. After the harvest in autumn, the tuber material must first be permitted to settle down to a certain state of inertia. The measurements cannot be carried out when, in spring, vegetation has begun. In both cases the unfitness of the material becomes evident by the fact that no constant values can be obtained. It is not possible to give an exact date of the beginning and the end of the "season." These dates depend not only upon the properties of the different varieties but also upon the sources of the seed. The degree of degeneration has to be taken into account also, as we were able to ascertain, particularly with some of the early varieties in which the degenerated tubers can no longer be measured, even from early in spring.

As already mentioned above, it became evident in the course of our investigations that not all varieties can be judged on an equal basis. We know today that there are several groups of varieties to which belong those most generally cultivated. In the first group we find the early variety of Erstling and Juliniere, furthermore, the Odenwälder Blaue, and the late variety of Wohltmann Broad Leaf, varieties that are by no means closely related to each other. The first group shows during winter dormancy the border line of healthy tubers at a potential value of calomel electrode minus 195 millivolts. A second group seems to comprise the majority of all varieties. To it belong the well known varieties Industrie, Direktor Johannsen, Parnassia, etc. During the period of winter dormancy, their vitality border lies at about Ec-165 mV. It is not yet certain whether there exists a third variety group, the border value of which lies at a different point. The variety Magnum Bonum, probably degenerated already at lesser values, seems to prove the existence of a third group. Although it is possible to say today that all potatoes, independent of the qualities of the varieties, are healthy if they show values up to electrode - 150 millivolts and diseased if the values are below electrode - 200 millivolts, the determination of the border values of the different varieties is of extreme importance in practice. Within the groups known to us, different sources of different varieties may be compared with regard to their cropping value. It is our aim to arrive at a comparison of the cropping values independently from the properties of the different varieties and to make such comparison on a known basis.

Of great importance, even for comparative measurements of the sources of one special variety, is the length of the measuring time. I have already mentioned that there is such a thing as season. This season, however, comprises a longer period than actual winter dormancy would require. The same basis of an immutable vitality border, however, exists in all varieties only at the time of complete inertia of the tuber, namely, during the actual

winter rest. Comparative measurements have shown that in autumn, when the possibility of obtaining constant values is given, the border values of the different varieties are at first somewhat higher. They then decline monthly by 10 mV. until winter rest begins, and remain, according to the species, at the same level for from 3 to 5 months, to drop again 10 mV. monthly in the spring of the year. Just as duration and temporal position of winter rest, so also the variability of the border values and their extent depend upon the properties of the different varieties.

You can see by these special questions that I have just discussed that Wartenberg and Hey, in their method of determining cropping values, have not quite found the solution of all problems. This is quite natural, since, in working on biological problems, out of every solved problem, new unsolved ones are arising. But, inasmuch as we are practical men, we are concerned to know how far the present methods are of practical value.

Determinations of the cropping value of seed that have been carried out in the Biologic Reich Institute with best results for practical use, have proved that this method is quite satisfactory even today. We divide the values into 3 groups: the vital group, the group of critical cases, and the group of degeneration. This division is somewhat more liberally defined than it is possible to determine the cropping values of seeds by actual measurement. This is of practical importance insofar as the effect of the cropping value not only depends on the state of health of the tubers, but also on the ecological conditions of the localities where they will be cultivated next year. The vitality group comprises potatoes, the potential values of which indicate perfect health. This may be ascertained, even today, with absolute certainty. With similar accuracy can be recognized seed material belonging to the degeneration group. The critical cases are critical in a double sense. First, their values lie near the border of vitality, so that it is rather difficult to judge whether the tuber is healthy or not, particularly if the border value of the variety in question has not yet been exactly determined. One must be careful in operating with cases approaching the border value, even if the latter be known; for the border value is but an expression of the transitionary zone. So, there will always be some uncertainty with regard to any judgment about values lying within the critical zone. This uncertainty increases because the ecological conditions of the cultivation ground may either augment the degeneration of the material, or inversely promote its recovery under favorable conditions.

What number of individual determinations is necessary in order to make the result of a test of one certain source conform to actual facts?

With a total number of 100 to 120 tubers, all possibilities and their frequency may be determined. This comparatively large number, therefore,

assures also the greatest certainty. A number of 50 or 60 tubers will show the types that may be expected, as well as a satisfactory indication of their frequency. The examination of only 20 to 30 tubers, however, enables one to say merely whether a certain seed material will produce degenerated plants. It may happen that the prognosis based on such a small number of tubers is faulty. But the probability of an error in experimenting with 30 tubers only is so small that even with this number, the question may be satisfactorily settled by the method of Wartenberg and Hey whether there is a considerable number of degenerated tubers among the material used in the determinations.

# A FUSARIUM DISEASE OF CEREUS SCHOTTII

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## INTRODUCTION

Besides the many species of *Fusarium* that have been reported as causing diseases of economic plants, there has been some work done on the relation of *Fusarium* spp. to diseases of certain ornamentals. *F. conglutinans callistephi* causes wilt in the China aster (3); *F. sp.*, a stem rot of aster (1); *F. dianthi* Prill. and Delac., a stem rot of carnation (2); *F. sp.*, wilt of carnation (7); and *Fusarium* rot, caused by *F. oxysporum* var. *gladioli*, is an important disease of gladiolus corms (4). The cacti, extensively used as ornamentals, are subject to various diseases. This paper deals with one of these diseases and the causal organism, which has been found to be a *Fusarium*.

## HOST

*Cereus schottii*, the host plant of the *Fusarium* studied, grows in Sonora (Mexico), southern California, and southern Arizona. The diseased plant from which the fungus was first isolated was brought from Sonora, near Magdalena. An identical *Fusarium* was later obtained from a plant growing in the cactus garden of the University of Arizona. Since the latter had been placed in the garden nearly a year before it showed signs of disease, it seems doubtful that it was infected when placed in the garden. Therefore, it appears safe to say that the disease occurs in the northern and southern limits of the area in which the host is found. No data are available as to whether or not the disease occurs in California.

## SYMPTOMS

The diseased plant from which the *Fusarium* was isolated showed a necrosis of the rot type. A large, slightly sunken lesion covered the entire lower half of the stem. This lesion was fuscous to fuscous black in color with a narrow area of light green yellow, 3 mm. in width, around the margin.

The interior of the infected part of the plant was colored fuscous to fuscous black. All the tissues were involved, so it was impossible to judge what part of the plant was first attacked or the manner in which the parasite entered and made its way through the plant.

<sup>1</sup> The writer wishes to express sincere appreciation to Dr. J. G. Brown, Head of the Department of Plant Pathology, University of Arizona, for his advice and assistance in this work. The writer is also indebted to Mr. M. M. Evans, Research Assistant in the Department, for his help with photographs appearing in this paper.

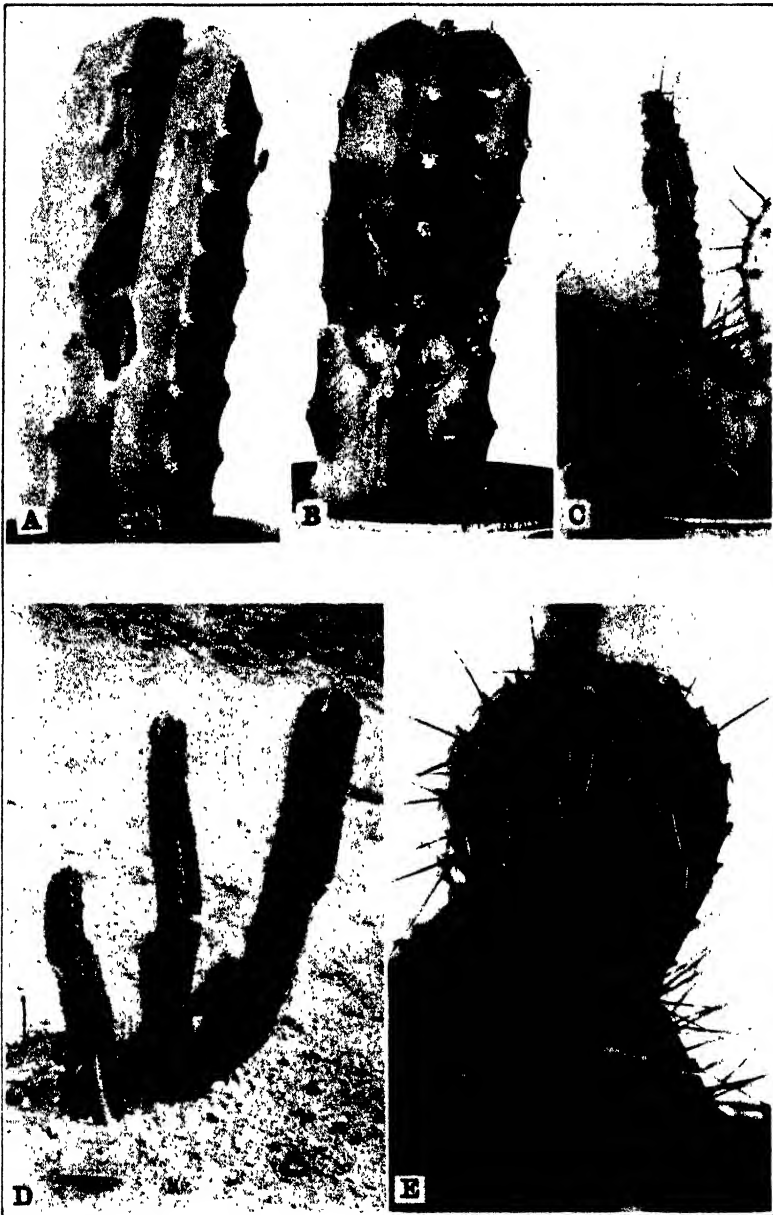


FIG. 1. A. Plant of *Cereus schottii* 10 days after inoculation, showing fuscous lesion with light-green margin. B. Same plant 24 days after inoculation. Corking off of lesion has begun. D. Plant of *C. schottii* from which the *Fusarium* was isolated. C. and E. Prickly pear (*Engelmannii* group), showing swelling and beginning of discolorations as result of inoculation. C. Profile of the swelling. E. Surface view.

## CAUSE

## a. Isolation

In the fall of 1931 a *Fusarium* was isolated from a plant of *Cereus schottii* (Fig. 1, D), in the following manner. The plant was broken open and bits of the diseased tissue cut out with a sterile scalpel and placed on slants of potato agar containing 2 per cent dextrose. In most cases a pure culture of the *Fusarium* was obtained. Since plants of *C. schottii* were not available at this time, the pathogenicity of the fungus was then determined by inoculating various species of *Opuntia*, chiefly of the *Engelmannii* group.

Monosporidial cultures were then obtained in the following way. A thin suspension of spores was made and kept at a temperature just above the solidification point. The agar containing spores was drawn up into a sterile capillary tube by means of a rubber syringe bulb. The contents of the tube, after being allowed to cool, were examined under the microscope and single spores separated from any others were marked. The pieces of glass tubing containing single spores were then cut out, dipped in 1-1000 mercuric chloride solution, and placed on agar slants. The cultures thus obtained were used for all future work.

## b. Reactions

1. *Potato—2 per cent Dextrose Agar, pH 7.2–28° C.* Mycelium was well developed. Aerial hyphae present, 3 to 5 mm. in height, of a loose, cottony appearance, becoming slightly more compact and appressed with age; pure white in young cultures, becoming a pale pinkish buff with age. Discoloration of the medium began in 7 to 10 days. In a 21-day culture, the medium was bordeaux to dahlia carmine. Hyphae within the medium were not colored. Blue black sclerotia were found in 3-day and older cultures.

Lowering the pH to 5.6 produced the same results as those given above, except that growth was somewhat retarded. Potato- 5 per cent and 10 per cent dextrose agar, pH 7.2, were used with same results as given above, except that growth was slightly increased.

2. *Autoclaved White Corn Meal, 28° C. (Fig. 2, I).* The growth was much like that on potato-dextrose agar but slightly more compact in young cultures and never over 3 mm. in height. Medium was colored magenta to dahlia carmine, beginning on the 5th day and reaching bottom of plate in 12 days. The rate of growth on white corn meal was as follows:

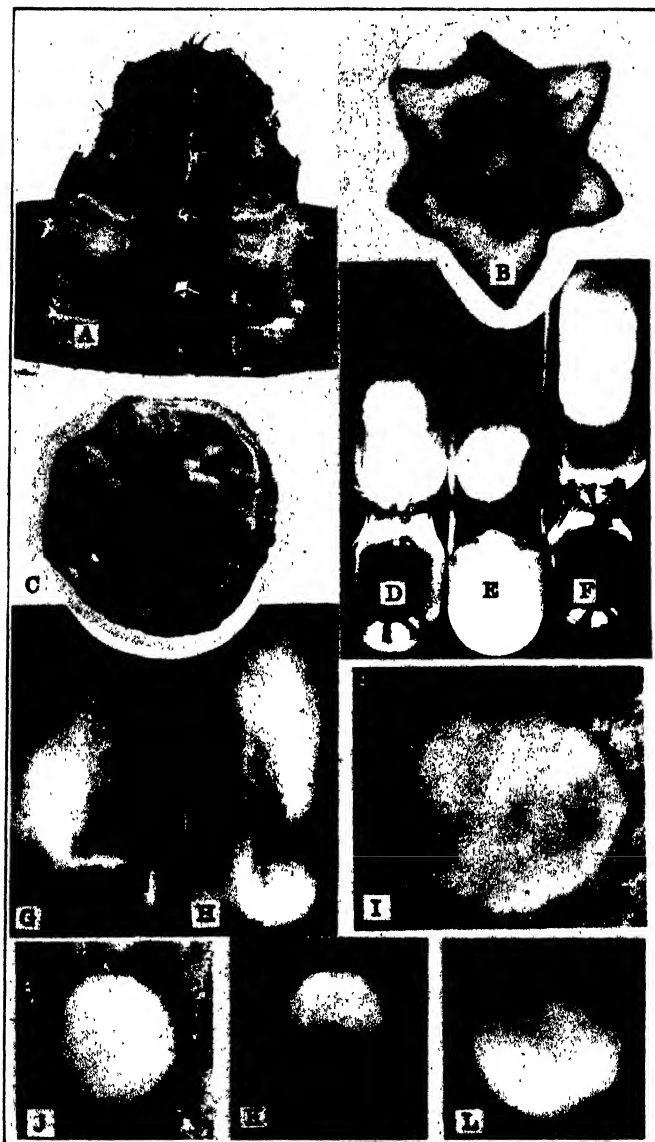


FIG. 2. A. Apex of plant of *Cereus schottii* three weeks after inoculation, showing discoloration. Cork tissue was not developed. B. Cross section of plant in A with discoloration of vascular system and tissues adjacent to it. C-L. 6-day cultures. C. On slice of living sweet-potato root.  $\times .5$ . D. On steamed potato plug.  $\times .79$ . E. On steamed carrot plug.  $\times .79$ . F. On steamed watermelon plug.  $\times .79$ . G. On steamed cotton stem.  $\times .88$ . H. On steamed watermelon stem.  $\times .88$ . I. On autoclaved white corn meal,  $27^{\circ}$  C.  $\times 1.12$ . J. On autoclaved rice,  $27^{\circ}$  C.  $\times 1.13$ . K. On oatmeal agar,  $27^{\circ}$  C.  $\times 1.12$ . L. On yellow corn meal,  $27^{\circ}$  C.  $\times 1.12$ .



Age of culture	Diam. of culture
24 hrs.	No perceptible growth
48 "	1 mm.
3 days	10 "
4 "	13 "
8 "	19 "
12 "	32 "
16 "	45 "

3. *Autoclaved Yellow Corn Meal*, 28° C. (Fig. 2, L). Same as white corn meal. Rate of growth slightly differed from above from day to day, but the growth over a period of 16 days was the same as given for white corn meal.

4. *Oatmeal Agar*, 27° C. (Fig. 2, K). Same as above.

5. *Steamed Rice*, 28° C. (Fig. 2, J). Growth more compact, less fluffy than on potato-dextrose agar. Pale hortense violet color of medium appeared around the edge of the colony after 5 days. In 14 days color reached bottom of Petri dish. Color of medium in 14-day culture, pale hortense violet to mulberry purple. In 60-day culture, the medium was a dull violet black. Hyphae within the medium, not discolored.

Rate of growth, slightly more rapid than that given for white corn meal. The growth after 16 days measured 5 cm. in diameter.

6. *Nutrient Agar*, pH 7.2, 28° C. Aerial mycelium well developed, 3-5 mm. in height, of a loose cottony appearance. Mycelium was pure white in young cultures. With age, growth appeared more compact and pale pinkish buff. No discoloration of the medium. Growth was slow on this medium as compared with that on potato-dextrose agar, yellow corn meal and white corn meal.

On nutrient agar, pH 5.6, growth was very slow and aerial mycelium not so well developed as at pH 7.2.

7. *Steamed Potato Plugs*, 28° C. (Fig. 2, D). Aerial mycelium much more compact, less fluffy than on any of the media described above. Growth was raised only slightly above the surface of the medium and was rather slow as compared with that on potato-dextrose agar. On the fifth day, medium became discolored a deep livid purple. By the 16th day the entire potato plug was colored deep livid purple, later turning dark violet gray.

8. *Autoclaved Watermelon Stem*, 28° C. (Fig. 2, H). On second day the growth appeared as a small tuft of erect, delicate, white mycelium from the point of inoculation. In 10 days erect, delicate hyphae spread over the surface of the stem and sides of the test tube; mycelium more compact than on 2-day culture but never so much so as on potato-dextrose agar, corn meal, or rice. Growth more rapid than on any other medium, except watermelon stem. Mycelium pale pinkish buff with age.

9. *Autoclaved Cotton Stem*, 28° C. (Fig. 2, G). Same as above.

10. *Czapek Agar*, 28° C. Growth slow as compared with potato-dextrose agar; appearance of culture as given for potato-dextrose agar; discoloration of medium beginning on seventh day, light neutral red.

11. *Two Per cent Glucose Agar*, 28° C. Appearance of culture much like that on potato-dextrose agar but slightly more compact; height of aerial mycelium not exceeding 3 mm. In 10 days medium was discolored tawny, later turning to ochraceous tawny and orange cinnamon. Aerial mycelium, pure white in young cultures turning pale ochraceous buff with age.

12. *Slices of Living Sweet-Potato Root*, 27° C. (Fig. 2, C). In 6 days most of surface was covered with white mycelium, not uniform in height, but ranging from 1 mm. to 5 mm. Mycelium turned light dove gray with age. Rot of tuber produced, fuscous in 21-day culture.

13. *Living Whole Potato Tuber*, 27° C. No growth.

14. *Living Whole Sweet-Potato Root*, 27° C. White mycelium from point of inoculation. After 5 days aerial mycelium became a light dove gray. Fuscous rot of tuber produced.

#### c. Microscopic Appearance of Hyphae

Hyphae from the various cultures described were examined in stained sections of a culture on potato-dextrose agar and in stained sections of the diseased stem of *Cereus schottii*. Sections were stained in Heidenhain's iron-alum haematoxylin and with light green in clove oil and saffranin. Hyphae were simply branched. The diameters of 25 vegetative cells from a culture on 2 per cent potato-dextrose agar averaged 2.43  $\mu$ . The average length of 25 such cells was 25.8  $\mu$ .

Tests with Sudan III and osmic acid on hyphae taken from a culture on autoclaved white corn meal showed numerous oil globules in most cells. A negative test for glycogen was given with potassium iodide. Cells that had most of their protoplasmic contents and had become extensively vacuolate were common in all cultures; only a thin layer of protoplasm remained around the cell wall in some cases. Swollen hyphal cells were numerous in all cultures. Some of these cells closely resembled chlamydospores, but they showed only a single wall (Fig. 3, A).

#### d. Reproduction

Microconidia and macroconidia (Fig. 3, E-G) were found in cultures. Microconidia, both ovoid and sickle-shape, were numerous in young cultures on all the media used. In the first cultures examined, nonseptate spores were produced almost exclusively on all media. When septate spores were transferred from sporodochia, which occurred occasionally, cultures pro-

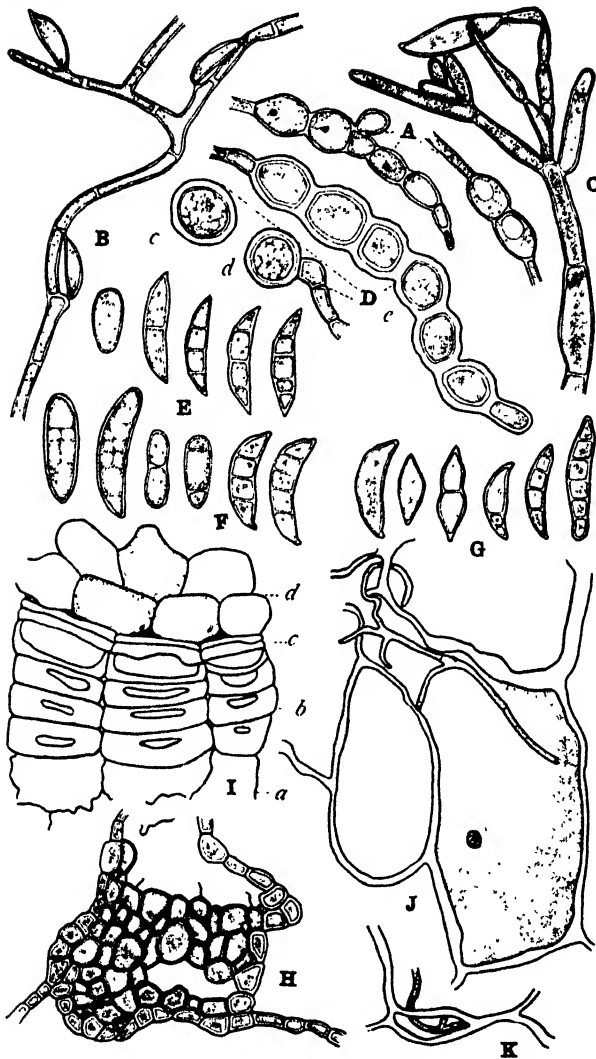


FIG. 3. A. Swollen hyphal cells from a 52-day-old culture on white corn meal.  $\times 1060.4$ . B. Part of a conidiophore from a 40-day-old culture on 2 per cent potato-dextrose agar, pH 7.0.  $\times 1060.4$ . C. Conidiophore from a 104-day-old culture on white corn meal.  $\times 1060.4$ . D. Group of chlamydospores: *c* and *d*, from a 42-day-old culture on 2 per cent potato-dextrose agar, pH 7.0.  $\times 1060.4$ ; *c*, chlamydospores in a chain from a 42-day-old culture on stemmed cotton stem.  $\times 1060.4$ . E. Group of spores from a 51-day culture on white corn meal.  $\times 1060.4$ . F. Group of spores from a 104-day culture on white corn meal.  $\times 1060.4$ . G. Group of spores from a 90-day culture on 2 per cent potato-dextrose agar, pH 7.0.  $\times 1060.4$ . H. Section of a sclerotium.  $\times 1060.4$ . I. Host tissue: dead parenchyma cells (*a*), cork cells (*b*), cork cambium (*c*), and normal cells (*d*).  $\times 154$ . J. Intracellular hypha.  $\times 330$ . K. Hypha in intercellular space.  $\times 330$ .

ducing septate spores were obtained. Microconidia were not borne in sporodochia nor pycnidia.

Macroconidia were produced in sporodochia that were confluent in nature. Conidial masses were pale vinaceous pink. Macroconidia were gradually attenuate at both ends, usually uniformly curved throughout; apedicellate, or with the foot poorly developed. Macroconidia were 3-5 septate.

Nonseptate chlamydospores, both terminal (Fig. 3, D, *d*) and intercalary (Fig. 3, D, *e*), were found in cultures on potato-dextrose agar, autoclaved potato plugs, corn meal, rice, and autoclaved stems. Although chlamydospores were found without difficulty, they were never abundant. The average diameter of 10 such spores, measured through the widest part of the spore, was 15.2  $\mu$ . Cells differing from true chlamydospores, in that they had only a single wall, were very numerous in all cultures (Fig. 3, A).

Blue black sclerotia were abundant (Fig. 3, H). In cultures 30-days old on potato-2 per cent-dextrose agar numerous sclerotia were found imbedded in the medium. In cultures 70 days old, on autoclaved rice and corn meal, sclerotia were so numerous that the remaining medium appeared blue black. Sclerotia varied in size up to 1½ mm. in diameter.

#### c. Identification

By comparison with the descriptions (6, 8) of the section *Elegans* of the genus *Fusarium*, it is evident that the fungus belongs in that group. The fungus described in this paper agrees with these descriptions in the presence of both microconidia and macroconidia; in the occurrence and size of terminal and intercalary chlamydospores and blue black sclerotia; in the degree of development and color of aerial mycelium and in the discoloration of media. However, 3-septate conidia are given in the descriptions as typical for this group while 4- and 5-septate conidia were found to predominate in 2 groups of 100 spores measured in this study.

Within the section *Elegans*, the fungus falls close to *Fusarium oxysporum*, but there are certain differences that appear to be of sufficient importance to set it apart as a variety of *F. oxysporum*. The fungus agrees with *F. oxysporum* in the following characteristics. Conidiophores are typically much branched (Fig. 3, B and C). Sickle-shape microconidia are gradually attenuated toward the apex, nearly cylindrical and broadest in the middle half of the length. Macroconidia are borne in sporodochia. Conidial masses are pinkish buff. Aerial mycelium is white and well developed, of medium height, 3-5 mm., and discoloration of the substrate varies through shades of red and purple. Both terminal and intercalary chlamydospores are present, and also blue black sclerotia.

Characteristics that do not agree with Wollenweber's (8) and Sherbakoff's (5) descriptions of *Fusarium oxysporum* are as follows. The nonsep-

TABLE 1.—*Spore measurements\* of organism obtained from diseased plants of Cereus schottii*

Medium	0-Septate	1-Septate	2-Septate	3-Septate	4-Septate	5-Septate
White corn meal 10-day	93 per cent 5.2-14.5 × 2.5-4 μ Av., 9.7 × 2.3 μ	6 per cent 15.5-16.5 × 3-4 μ Av., 15.3 × 2.3 μ	1 per cent 15.5-16.5 × 3-4 μ Av., 15.5 × 3.5 μ			
White corn meal 43-day	14 per cent 8-12 × 2-3 μ Av., 9.8 × 2.8 μ	18 per cent 8.5-9.5 × 2.5-3.5 μ Av., 8.8 × 2.7 μ	60 per cent 8-11 × 2.5-3 μ Av., 9.6 × 2.6 μ	8 per cent 9-12 × 2.8-3 μ Av., 10 × 2.5 μ		
2 per cent potato-dextrose agar pH 7.2	83 per cent 4.2-11.5 × 2-3 μ Av., 9.9 × 2.7 μ	4 per cent 9-14.2 × 2.5-4 μ Av., 11.5 × 3.8 μ	13 per cent 8-14.8 × 3-4 μ Av., 12.2 × 3.5 μ			
20-day						
2 per cent potato-dextrose agar pH 7.2	4 per cent 9-12.5 × 2.8-3.9 μ Av., 11.8 × 3 μ	10 per cent 9-15.5 × 2.8-4 μ Av., 14.8 × 3.6 μ	4 per cent 9-16.8 × 3.2-4 μ Av., 15.2 × 3.6 μ	21 per cent 11.5-16 × 3-4 μ Av., 15.5 × 3.5 μ	9 per cent 14.2-17.2 × 3.2-4 μ Av., 15.2 × 3.5 μ	52 per cent 17.2-21.4 × 4-4.2 μ Av., 19.9 × 4.15 μ
63-day						
Autoclaved cotton stem 32-day	5 per cent 10.5-13.5 × 2.5-3 μ Av., 12 × 2.7 μ	16 per cent 9-16 × 3.1-3.5 μ Av., 11.9 × 3.1 μ	7 per cent 10.5-16 × 2.8-3 μ Av., 14 × 2.9 μ	72 per cent 11.5-16.5 × 2.5-3.5 μ Av., 14.5 × 3 μ	(50 spores) 8 per cent 14.2-15 × 3-4 μ Av., 14.4 × 3.9 μ	62 per cent 16-17.2 × 4-4.2 μ Av., 16.3 × 4 μ
Autoclaved watermelon stem 51-day	5 per cent 11-12.9 × 2.8-3.5 μ Av., 12.5 × 3.2 μ	6 per cent 9-16 × 2.8-4 μ Av., 15.3 × 3.9 μ	4 per cent 9-16.2 × 3.4-4 μ Av., 14.2 × 3.4 μ	15 per cent 11-16 × 3-4 μ Av., 15.4 × 3.9 μ		

\* Measurements are for 100 spores, except as indicated.

tate microconidia measured, taken from a culture on 2 per cent potato-dextrose agar, pH 7.0, averaged  $9.9 \times 2.7 \mu$  ( $4.2-11.5 \times 2-3 \mu$ ) as compared to  $8 \times 2.9 \mu$  ( $4.5-12 \times 2-4 \mu$ ). One-septate spores averaged  $11.5 \times 3.8 \mu$  ( $9-14.2 \times 2.5-4 \mu$ ) compared to  $16.5 \times 3.4 \mu$  ( $11-23 \times 2.5-4.1 \mu$ ). Two-septate spores averaged  $12.2 \times 3.5 \mu$  ( $8-14.8 \times 3-4 \mu$ ) compared to  $18.5 \times 3.9 \mu$  ( $17-26 \times 3-4.2 \mu$ ). Three-septate spores averaged  $12.2 \times 3.5 \mu$  ( $8-14 \times 3-4 \mu$ ) compared to  $27.5 \times 4 \mu$  ( $15-39 \times 3-4.7 \mu$ ).

Four-septate conidia occur only rarely, according to the descriptions of *Fusarium oxysporum* previously mentioned, never making up more than 3 per cent of the number of spores measured, while 8 and 9 per cent measured in 2 groups of 100 each in this study were 4-septate spores averaging  $14.4 \times 3.9 \mu$  ( $14.2-15 \times 3-4 \mu$ ) and  $15.2 \times 3.5 \mu$  ( $14.2-17.2 \times 3.2-4 \mu$ ), respectively, compared to  $36.7 \times 4.43 \mu$  ( $34.5-60 \times 4.3-4.5 \mu$ ). In the descriptions, 5-septate conidia make up 1 per cent of the spores measured, while in the 2 groups of 100 spores here studied the 5-septate spores predominated, the average size being  $16.3 \times 4 \mu$  ( $16-17.2 \times 4-4.2 \mu$ ) and  $19.9 \times 4.15 \mu$  ( $17.2-21 \times 4-4.2 \mu$ ) as compared to  $35 \times 4.4 \mu$  (no minimum and maximum given).

The spores of the fungus studied were either apedicellate or had the foot typically less well-developed than is characteristic of *Fusarium oxysporum*. There also is a marked difference in the parasitism of the 2 organisms. The *Fusarium* studied does not infect the Irish potato.

Therefore, because of the minor differences between *Fusarium oxysporum* and the organism described in this paper, the differences in spore size, the poor development of the foot, and the difference in parasitism, it would seem that the organism might be classified as a variety of *F. oxysporum*.

#### f. Effect Upon the Host

Inoculation of plants of *Cereus schottii* and prickly pear were made by cutting a small slit in the sterilized surface of the stem with a sterile scalpel and inserting a bit of monosporidial culture. Within 10 days a pronounced swelling appeared around the point of inoculation (Fig. 1, C and E). The raised area was discolored fuscous with a light green yellow margin. In 6 days the lesion had become sunken and had doubled in size. (Fig. 1, A, B). After 3 weeks, some of the plants had become fuscous or fuscous black almost throughout, while others had checked the progress of the organism by developing several rows of cork cells between the lesion and the normal tissue (Figs. 2, A, and 3, I). These thick-walled cork cells originated in the parenchyma.

Upon examination of plants that did not develop protective tissue the vascular system and parenchyma adjacent to it were found to be discolored the entire length of the stem. Stained sections of tissue at some distance

from the point of inoculation showed no hyphae present. These observations led to the conclusion that the fungus was excreting a substance or substances toxic to the living plant cells and that the toxic excretion was carried through the plant in the vascular system.

To further investigate this condition, a culture of the *Fusarium* was grown in nutrient broth containing 2 per cent peptone (6). After 8 days, the broth was filtered so that all spores of the fungus were excluded and the filtrate examined under the microscope to be certain of this fact. A cut end of a stem of *Cereus schottii* was then immersed about  $1\frac{1}{2}$  inches in the filtrate and allowed to remain there for 8 days. The stem was then cut longitudinally and examined. The first  $\frac{1}{2}$  inch of stem above the cut surface was discolored fuscous throughout. Above this region only the vascular bundles were discolored a light brown. This discoloration of vascular tissues extended to the apex of the plant.

A cross section of the stem in the region of inoculation showed discoloration throughout. Sections of tissue in this region stained with Heidenhain's iron-alum haematoxylin showed the presence of both intercellular (Fig. 3, J) and intracellular (Fig. 3, K) hyphae. In the older part of the lesion, cells had entirely lost their protoplasmic contents and cell walls were badly broken down. Some sections showed only hyphae of the fungus and fragments of cell walls.

#### SUMMARY

A *Fusarium* was isolated from a plant of *Cereus schottii* which showed necrosis of the rot type. Inoculations proved the fungus to be pathogenic. Monosporidial cultures were then made.

The *Fusarium* was found to belong to the section *Elegans* and to fall close to *F. oxysporum* within that group. It differs from *F. oxysporum* in having smaller macroconidia, a higher percentage of 4 and 5-septate spores, in having spores with the foot less well-developed than is characteristic of *F. oxysporum*, and in its pathogenicity. For these reasons the fungus is tentatively classified as a variety of *F. oxysporum*.

When plants of *Cereus schottii* were inoculated with the *Fusarium*, symptoms were produced that were identical with those shown by the naturally diseased plants, and the fungus was reisolated.

Inoculated plants of *Cereus schottii* showed discoloration of the vascular system and adjacent cells. Discoloration of tissues extended beyond the cells containing hyphae of the fungus as shown by stained sections. This fact led to the conclusion that a substance (or substances) toxic to the cells of the host was being excreted by the fungus. The *Fusarium* was grown for 8 days in nutrient broth. The broth was then filtered and the cut end of a stem of *Cereus schottii* immersed in the filtrate. The results of this

experiment substantiate the belief that a toxic substance was excreted that affected the living cells of the host.

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# EFFECT OF LIGHT ON THE DEVELOPMENT OF THE UREDIAL STAGE OF PUCCINIA GLUMARUM<sup>1</sup>

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## INTRODUCTION

Considerable difficulty has been experienced in propagating stripe rust, *Puccinia glumarum* (Schmidt) Eriks. and Henn., in the greenhouse at Moscow, Idaho. In the earlier work of Hungerford and Owens (3), at Corvallis, Oreg., very little if any difficulty was encountered in propagating this rust under greenhouse conditions. Although the reason for this difference is not definitely known, it is thought to have been due to the difference in climatic conditions. It was, therefore, deemed fundamental and necessary to the undertaking of any intensive study of this fungus to determine the conditions necessary for its optimal development in the greenhouse. An investigation of this type, where light and temperature could be controlled, was necessary also before cultures of stripe rust could be satisfactorily carried through the hot, dry summer months. The object of this work, therefore, was (1) to determine the effect of photoperiodism on *P. glumarum*; (2) to determine the optimum light intensity for the best development of the fungus; (3) to ascertain whether successful infection could be secured by subjecting the plants to light immediately after inoculation without first placing them in the shade for 48 hours; and (4) to determine whether sporulation could be secured from plants inoculated with urediospores that had been developed under a low light intensity.

Wilhelm (9), in a study on the mode of specialization of the urediospores of *Puccinia glumarum*, found that a reduction in light intensity prevented full expression of the type of infection but did not affect the relative susceptibility of the different varieties. Stakman and Levine (7) and Peltier (6), working with *P. graminis* (Pers.), and Fromme (1), working with *P. coronifera* (Kleb.), secured corresponding results, viz., these cereal rusts

<sup>1</sup> Cooperative investigations between the Idaho Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

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<sup>3</sup> The writer wishes to thank Dr. C. W. Hungerford, Plant Pathologist of the Idaho Agricultural Experiment Station, for his hearty cooperation during the progress of this work, and also to acknowledge his indebtedness to Dr. H. B. Humphrey, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for helpful suggestions in the preparation of the manuscript, and to Miss Ruth Remsberg, Assistant Pathologist, Idaho Agricultural Experiment Station, for help with the photographs.

developed more luxuriantly in fairly high light intensities than they did in lower ones. Melander (4), working with *P. graminis tritici*, form 15, found that extremely high light intensities, as well as low ones, caused a reduction in the ability of the urediospores to withstand low temperatures.

#### EXPERIMENTAL METHODS AND RESULTS

The method of inoculating the plants was, with certain modifications, the same as that employed by Stakman and Piemeisel (8, p. 432) in their work with *Puccinia graminis*. A spore suspension was first made by placing a drop of water on an ordinary glass slide and then, with a scalpel, scraping the spores from an infected plant into the drop of water. A small flattened needle was used to apply the spores on the primary leaf, only. Immediately following inoculation the plants were covered with a bell jar for 48 hours. In this method it was found unnecessary to draw the leaf between the thumb and forefinger before inoculation to remove the waxy coating, or to atomize the leaves after the spores had been applied. Sufficient spore material was used for each inoculation to insure good infection. The plants were kept trimmed down to the inoculated leaf throughout the investigation. The tests were performed in a temperature-control room, completely devoid of daylight. Stock cultures were maintained in the greenhouse by means of artificial light. Pannier, C. I.<sup>4</sup> No. 1330, a variety of barley known to be completely susceptible to *P. glumarum*, served as the host plant for the study herein reported. Single-spore cultures were not employed because the work was primarily undertaken for the purpose of studying the development of the fungus and not its specialization. All inoculations, however, were made from a culture of *P. glumarum* collected at Moscow, Idaho, in the fall of 1932.

The experiments were carried on in a temperature-controlled room 6 ft. x 9 ft. x 8 ft. Three ordinary Mazda 200-watt and 2 100-watt bulbs, with a voltage of 115, provided the light for the room. The lights were placed 30 inches from the plants, admitting illumination only from above. Ordinary Westinghouse daylight bulbs were used in place of the above, with no difference in results.

#### PHOTOPERIODISM

In the study of photoperiodism, 35 6-inch pots containing 5 plants each, or a total of 175, were inoculated as described above. At the termination of the infection period (48 hours after inoculation) the pots were grouped, so that there were 7 sets of 5 pots each. The inoculated plants were exposed, respectively, to 6, 8, 10, 12, 15, and 24 hours of light per day under identical temperature conditions. In other words a set of 5 pots was ex-

<sup>4</sup> C. I. denotes accession number of the Division of Cereal Crops and Diseases.

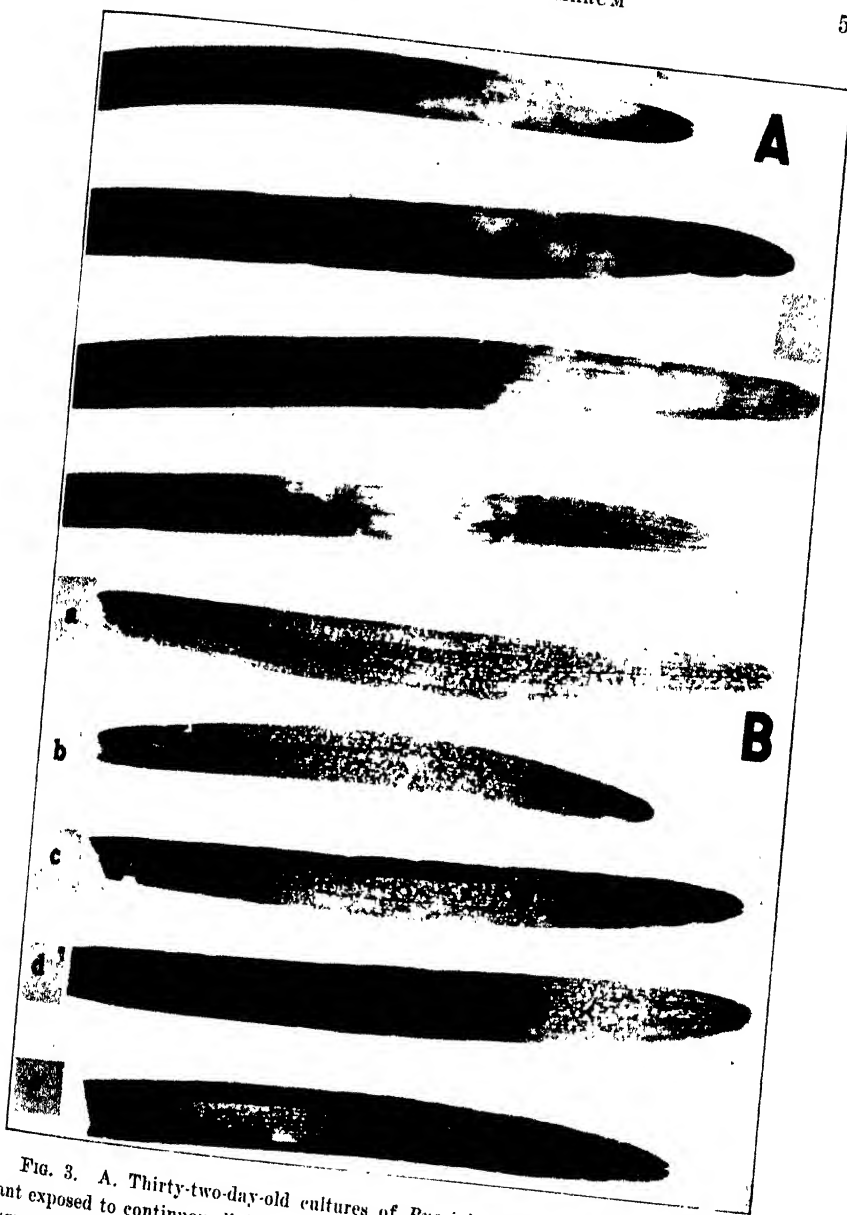


FIG. 3. A. Thirty-two-day-old cultures of *Puccinia glumarum* on leaves of barley plant exposed to continuous light at 45–50° F. B. Twenty-eight-day cultures of *Puccinia glumarum* on leaves of barley, showing the effect of light intensity on the subsequent development of the rust fungus: a, culture grown in light intensity of 960 foot candles; b, of 576; c, of 384; d, of 192; and e, of 96.

Light intensity has essentially the same effect on the development of urediospores of *Puccinia glumarum* as does the day length.

Table 2 shows in detail the results of the light-intensity studies.

Table 2 and figure 3, B show the effect of light intensity on the development of *Puccinia glumarum*. As in the case of the day length, the light intensity has its corresponding effect on the incubation period; that is, the higher the intensity, the shorter the incubation period. The light intensities employed in these investigations, however, did not change the rust type as did the longer day length. In general, these results agree with those of Stakman and Levine (7), Peltier (6), and Fromme (1).

In studying *Puccinia glumarum* in the greenhouse it has been the general practice to cover the newly inoculated plants with a bell jar and place them in a rather dark, shady place for 48 hours. This was done primarily to keep the humidity sufficiently high for germination and infection. It was thought that time and space could be conserved if the newly inoculated plants were exposed to the light immediately after inoculation. For this test 25 such plants were covered with bell jars and immediately placed in a compartment of medium light intensity. Other inoculated plants were placed in semi-darkness as controls. The bell jars were removed from all of the plants at the end of 48 hours.

Excellent infection was secured by subjecting the plants to light immediately after inoculation, a fact that is quite contrary to earlier teaching that plants inoculated with *Puccinia glumarum* should first be kept in a shaded place for 48 hours before subjecting them to the desired conditions. To obtain infection, however, it is necessary to cover newly inoculated plants with bell jars for 48 hours following their inoculation. Knowledge of this fact will enable one to conserve both time and space in one's study of this fungus. It will be especially helpful in varietal testing in the greenhouse, as well as in the study of physiologic forms.

Wilhelm (9), in his work on *Puccinia glumarum*, found that urediospores obtained from plants deprived of sufficient light were not viable and, therefore, were incapable of causing infection. In a similar study the writer inoculated 25 plants with urediospores that had been developed under a low light intensity (96 foot candles). These inoculated plants were placed in a compartment of higher light intensity (576 foot candles) and left for 15 days. Check plants were inoculated and placed in semi-darkness.

Contrary to the results of Wilhelm (9), good infection resulted when plants were inoculated with urediospores that had been developed under low light intensity (light produced by a single 100-watt lamp). No germination was secured in water cultures of urediospores of *Puccinia glumarum* that had been formed in such low light intensity. From previous experiences,

however, the percentage of germination of urediospores in culture is no positive criterion as to whether infection can or cannot be secured when plants are inoculated with urediospores that fail to germinate in culture.

In all of the above described tests, the temperature range was from 45° to 50° F. In no case was any special attempt made to regulate the humidity, although a record of the humidity was kept by means of a hygrothermograph.

#### DISCUSSION

It has been observed from time to time that the spring advent of *Puccinia glumarum* is very tardy. In checking over the weather records and the spring-advent dates of *P. glumarum*, it was found that in an unusually cloudy and rainy spring stripe rust appeared very late. The temperatures recorded for corresponding years of considerable cloudy weather did not vary sufficiently to explain the difference between advent dates of a cloudy spring and those of a sunny one. Therefore, in the light of the results herein presented, it would be safe to presume that in the case of considerable cloudy weather, the light intensity may be sufficiently decreased to delay the appearance of *P. glumarum*. This late initial appearance of stripe rust does not occur in springs characterized by a large percentage of sunny days.

#### SUMMARY

The day length, up to a certain number of hours, caused no change in the expression of the infection type of *Puccinia glumarum* at temperatures of 45° to 50° F. and 55° to 60° F. The shorter day increased the necessary incubation period.

The 6-hour day, compared with the 12-hour day, increased the incubation period 9 days. There was no further change in the period of incubation when the day length was increased beyond 12 hours.

When the day length exceeded 12 hours, there was a distinct change in the infection type from type-4 to type-0 (Fig. 2).

A high light intensity decreased the period of incubation and increased the subsequent development of the fungus.

Low light intensity did not permit the full expression of the rust; there was, however, no change in the infection type.

There was essentially no difference in the development of the fungus in the light intensities supplied by 400-watt and 600-watt bulbs.

Excellent infection was secured by subjecting plants to the lights immediately after inoculation rather than shading them for 48 hours before placing them under the lights. In all cases it was necessary to place a bell jar over the newly inoculated plants for the 48-hour period, to maintain the relative humidity necessary to spore germination.

Urediospores that were produced under a low light intensity failed to germinate in culture. When plants were inoculated with such spores, good infection resulted.

No attempt was made to control the relative humidity. It ranged between 58 and 92 per cent, as indicated by hygrothermograph records.

There was no difference in results in temperatures of 45° to 50° F. and 55° to 60° F. In temperatures of 68° to 70° F. the infection type was changed from a type-4 to a type-2 or 3. No infection was obtained at 80° F. or above.

No germination was secured in cultures of urediospores of *Puccinia glumarum* that had developed under low light intensity. Good infection, however, was obtained when plants were inoculated with spores from the same lot.

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# A SURVEY OF POTATO SCAB AND FUSARIUM WILT IN WESTERN NEBRASKA<sup>1</sup>

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## INTRODUCTION

The two most serious diseases of the potato in the commercial seed-producing sections of western Nebraska are potato scab (*Actinomyces scabies* (Thax.) Güssow) and Fusarium wilt and stem-end rot (*Fusarium cumartii* Carp. and *F. oxysporum* Schlecht.). Satisfactory methods of controlling these diseases have not been developed for the reason that most of the infection comes from the soil.

As a preliminary step in the investigations of the effect of various soil factors upon the occurrence of these two diseases the author undertook a survey of fields in western Nebraska in the summer of 1928. While the chief aim of the survey was to obtain data that would aid in outlining future experimental work, some of the records were thought to be of general interest and are here presented.

## SURVEY METHODS

In order to eliminate variation caused by infected seed tubers, 100 bushels of Bliss Triumph certified seed were selected from a single lot of seed potatoes and treated with hot formaldehyde (1 to 120) for 4 minutes at 124° F. One bushel of this treated seed was then sent to each of 100 growers of certified potatoes in western Nebraska. Each grower planted the bushel in one or more rows through the middle of a commercial field, otherwise planted to commercial certified seed treated with hot formaldehyde. The fields were selected as representing the widest range of environmental conditions existing in the potato-growing sections of the western 9 counties of the State. All but 4 fields were on dry-land in the high plains area, and these 4 exceptions were irrigated fields in the Scotts Bluff section.

*Field Records.* The following data were recorded for each field: soil type, the number of years the soil had been under cultivation, the crops grown for the preceding 5 years, and any such special treatments as manure, the preparation of the land, and number of cultivations and dates of planting and harvesting.

*Disease Records.* At harvest time each field was visited and a 100 pound random sample was taken from the rows used for the test. All

<sup>1</sup> Paper No. 142 of the Journal Series of the Nebraska Agricultural Experiment Station.

potatoes, regardless of size, were examined individually for scab and stem-end rot or vascular discoloration. The disease grades were as follows: *Scab Grades*; *Healthy*; *Slight*, i.e., not more than 1 spot  $\frac{1}{4}$  inch in diameter or 5 spots  $\frac{1}{8}$  inch in diameter; *Medium*, i.e., more than *Slight* but less than  $\frac{1}{4}$  of the surface of the tuber; *Severe*, i.e., more than  $\frac{1}{4}$  of the surface. Superficial scab occurring without definite corky lesions was also recorded. In this paper the term total scab refers to all of the above 4 types or degrees of severity.

*Fusarium Grades*:—*Healthy*; *Slight*, i.e., not more than  $\frac{1}{4}$  inch deep; *Medium*, i.e.,  $\frac{1}{4}$  inch to  $\frac{1}{4}$  of the length of the tuber; *Severe*, i.e., more than *Medium*. In addition to the above notes the type of infection also was recorded as vascular discoloration, stem-end rot, or a combination of these 2 types. Throughout this paper the term *Fusarium wilt* will be applied to tubers showing symptoms of the above types. All disease records were made on the basis of weight rather than number.

*Weather Data.* Temperature and rainfall records were obtained from the United States Weather Bureau records for 8 stations located within the survey area. In addition, rain-gauges were used at five other points in this area.

*Soil Data.* In late July and early August each field was visited and 2 sets of soil samples were taken, one for the determination of soil type, organic matter, and hydrogen-ion concentration,<sup>2</sup> and the other for the determination of the numbers of bacteria and Actinomyces and other fungi. The latter samples were taken in the row and between the hills with a 2-inch sterile tube to a depth of 6 inches, the loose surface soil first being removed. Three composite samples of 5 cores each were obtained from each test row. After thorough mixing a portion of each sample was placed in a sterile pint jar and the jars were immediately shipped to Lincoln, where they were either plated at once or placed in a cold-storage room held at a temperature of 36° F. Most of the samples were plated within a few days but it was necessary to hold some of them for a longer period of time up to one month. As it turned out, this introduced a considerable error when individual fields were compared<sup>3</sup> but the average storage periods of the samples as grouped for the tables that follow are practically the same.

<sup>2</sup> The writer is indebted to J. C. Russel of the Agronomy Department who cooperated in the collection of the samples and supplied the data on soil type, organic matter, and hydrogen-ion concentration. The latter were determined by the quinhydrone method.

<sup>3</sup> Because of the necessity of delay in plating, a parallel study of the effect of holding a sample in cold storage was undertaken. It was found that, when a sample was kept air-tight (in cold storage) and opened for plating at the end of 7, 14, and 55 days and for longer periods, the total number of organisms and the percentage of bacteria increased up to 14 days' storage and thereafter decreased, while the reverse was true of Actinomyces and other fungi.



It is realized that one sampling of a soil does not give a very clear picture of the occurrence or activities of the microflora. \*It was thought, however, that some evidence might be obtained regarding the relative prevalence of the 3 large groups of soil microorganisms and a possible indication of the relation of their occurrence to the amount of disease. Because of the amount of labor involved it was found impossible to plate out all samples, 231 samples from 77 fields were plated. Waksman's egg-albumen agar was used for determinations of numbers of bacteria and Actinomyces, and Waksman's acid fungus agar for counts of fungi. Ten plates of each agar were used for each of the 3 samples from each field.

Soil moisture determinations were made on each sample at the time of plating. The data given in the tables are on a dry weight basis.

In addition to the samples obtained from the potato fields, 9 samples were also taken from virgin soil identical in type to the potato fields adjacent.

#### RESULTS

*Prevalence of the two Diseases.* Scab was of common occurrence throughout the entire area surveyed and was quite severe in many fields. There were no fields where the potatoes were entirely free from either *slight* or *medium* scab, and *severe* scab was present in 67 per cent of the fields. The infection in all samples averaged 10.5 per cent *slight*, 12.5 per cent *medium*, and 1.4 per cent *severe* scab, with an additional 30 per cent of a superficial or russet type of scab. The percentage of scab severe enough to require sorting out is best approximated by combining the *medium* and *severe* scab. There were 75 fields with less than 10 per cent of combined *medium* and *severe* scab, 21 fields with 10 to 25 per cent, 12 fields with 25 to 50 per cent, and 7 fields with over 50 per cent. The occurrence of potato scab in this area is clearly shown to be of major importance and, primarily, a problem of soil infection.

The data on Fusarium wilt showed this disease to be almost as prevalent as scab. There were only 7 of the 118<sup>4</sup> samples of potatoes that were entirely free of stem-end rot or vascular discoloration. The average amount of infection for all fields was 4.5 per cent with about half, or 2.4 per cent of this being of a severe type. There were 9 samples with 10 to 20 per cent, and one each with 30, 33, and 47 per cent. The latter was from a field that had been under cultivation for only 9 years and had never before been planted to potatoes. The sample containing 30 per cent infection was grown on virgin soil. The type of infection in the tubers was chiefly a vascular discoloration and half of this was of a severe type and occurred

\* A few of the fields were divided into two samples because of differences in cultural practices, which accounts for the 18 samples in excess of the 100 originally planned.

as wide bands of discolored tissue extending more than one-fourth of the length of the tuber. There was very little stem-end rot without an accompanying vascular discoloration and in the combined form the severe types predominated.

The importance of the *Fusarium*-wilt problem is not fully indicated by the above data. It must be remembered that most of these infected fields contained wilted plants in the field and that many of these plants wilted early and did not produce tubers. The disease unquestionably resulted in reduced yields and more unmarketable tubers but in this survey it was not possible to keep a record of wilted plants in the field nor to obtain accurate yield records that would be comparable. Here, as with the scab, we have a problem that is primarily one of soil infection.

A summary of the commercial fields inspected for certification in this area the same year shows also the severity of these two diseases. Eighty-five per cent of the 530 fields inspected contained *Fusarium* wilt and these infected fields averaged 1.4 per cent, while 84 per cent of the lots of tubers showed infection in the bin inspection and averaged 2.5 per cent. It must be remembered also that diseased plants often were rogued out of the fields before inspection and that severely infected fields were disqualified before the bin inspection. Bin inspection showed that 73 per cent of the fields yielded scabby potatoes, averaging 10.1 per cent of infected tubers that would have to be sorted out of a U. S. No. 1 grade.

*Effect of the Preceding Crop.* The variation in the types of rotations or crop sequences used over a 5-year period was so great that only the crop immediately preceding potatoes was considered (Table 1). There was little difference in the amount of scab following wheat, oats, rye, and barley and these grains were all grouped together. There was more scab, both total and severe, following corn and summer fallow than following the small grains, with the legumes falling between these two groups. It must be remembered that there was considerable variation in each group as indicated by the probable errors in table 1 and that definite conclusions cannot be drawn in the groups containing only a few fields, as in the case with the legumes and summer fallow. There is, however, a clear indication that cultivation of fields the year preceding potatoes tends to increase scab as contrasted with noncultivated crops. The fact that no significant differences occurred with the different small grains and that summer fallow increased scab similarly to corn indicates that the cultivation rather than the crop itself was the significant factor.

In one field of oats the grower had burnt off the stubble on one-half of the field. The test row was placed with one-half on each part of the field. The oat stubble not burned produced 75 per cent total scab and 2.8 per cent *Fusarium* wilt in the tubers, while that part of the field with the

TABLE 1.—The effect of the crop immediately preceding potatoes upon the occurrence of scab and *Fusarium wilt* and upon the number and percentages of microorganisms.

Crop preceding potatoes	Disease records			Microorganisms per gram of soil				
	Number of fields	Total scab per cent	Medium <sup>b</sup> and severe scab per cent	Fusarium per cent	Number of fields	Total number of organisms, (000 omitted)	Bacteria per cent	Actino-myces per cent
Small grains	59	51.2 ± 2.4 <sup>c</sup>	13.1 ± 1.2	4.8	33	43,699	94.6	5.3
Alfalfa or sweet clover	7	60.5 ± 4.1	12.2 ± 2.5	2.4	5	39,069	94.4	5.5
Corn	18	62.1 ± 4.3	22.5 ± 4.7	3.9	8	40,667	93.8	6.1
Fallow	3	80.0 ± 4.9	27.2 ± 9.2	3.0	2	36,392	92.7	7.2
Virgin soil	1	67.0	4.3	29.5				.....

<sup>a</sup> All figures are based on the weight of infected tubers.  
<sup>b</sup> For definition of scab types see p. 518.  
<sup>c</sup> The probable error was determined by the following formula:

$$\sqrt{\frac{\sum(x^2)}{n} - \left[\frac{\sum(x)}{n}\right]^2} \times .6745 = PE_s.$$
$$\frac{PE_s}{\sqrt{n}} = P. E. \text{ of mean.}$$

stubble burned off produced only 47 per cent scab and no wilt. The soil was very uniform and, other than for the burning, the field was handled as a unit, so that both plots received similar treatment.

In regard to *Fusarium* wilt there were few outstanding differences. The smallest amount of wilt occurred following the legumes. In the fields following small grains there was a wide variation in the percentage of wilt, indicating that factors other than the preceding crop were probably of great influence. There was nothing to substantiate the common opinion of many growers that wilt is worse on corn land.

The fields grouped according to table 1 failed to show any correlation between disease and either organic matter or H-ion concentration. Plate cultures from 48 of these fields showed a higher average percentage of *Actinomyces* in the fallow and cornfields than in the small grains. The largest number of *Actinomyces* was in the fallow soil. The higher percentage of *Actinomyces* was accompanied by a lower percentage of bacteria and a slightly higher percentage of fungi. The average total number of all 3 groups of organisms was least in the fallow and highest with the small grains.

*Interval between Potato Crops.* A common recommendation to potato growers is to allow a period of time, usually 4 or 5 years, to elapse between potato crops. In view of the fact that virgin soil produced a large amount of scab (Table 1) the data were examined to determine the effect of the length of time between crops of potatoes upon the amount of scab and *Fusarium* wilt.

None of the fields had been planted with potatoes during the previous 2 years, so it was impossible to determine the effect of a recent crop of potatoes. The data are shown in table 2.

TABLE 2.—*The effect of the interval between potato crops upon the amount of scab and Fusarium wilt*

Interval between potato crops	Number of fields	Total scab per cent	Medium and severe scab per cent	<i>Fusarium</i> per cent
2 to 4 years .....	18	78.5	31.4	3.4
More than 4 years ..	10	56.6	12.1	5.0
No potatoes .....	61	46.2	9.8	5.3

The highest percentage of scab was for the interval of 2 to 4 years. There was a sharp drop, however, between this group and the group with an interval longer than 4 years and a further reduction in those fields that had never been planted to potatoes.

These data show that while intervals of more than 4 years between potato crops tend to reduce scab, that time alone is not sufficient to eliminate the disease or even to prevent serious loss.

The above conclusions do not apply to *Fusarium* wilt. In fact they are apparently the reverse. As shown in table 1, one of the most severely infected fields was planted on virgin soil.

*Effect of Manure and Legumes.* It is often stated that the addition of manure increases the amount of scab and that the use of a green manure in the form of alfalfa will reduce scab.

In this survey only 6 fields had ever received an application of manure and none of these within 2 years. Two that had never been planted to potatoes or alfalfa produced 31 per cent infected tubers, while the other 4 that had grown potatoes previously, produced 85 per cent infection. Alfalfa preceded potatoes in 9 fields that had never been planted to potatoes or received manure, and these fields produced 41 per cent infected tubers. Only 7 fields that had never been planted to either potatoes or alfalfa or received manure averaged 48 per cent infection of scab.

The effect of previous potato crops was more pronounced than the effect of either manure or alfalfa, but there was no indication in these few fields that the addition of manure in previous years had resulted in more scab but rather the reverse, as less scab occurred than following alfalfa. The number of fields involved, however, is too small to warrant definite conclusions.

*Soil Reaction.* It has frequently been stated in the literature that low pH values result in decreased scab and that a pH of about 5.0 to 5.4 is the desirable range for scab control and increased yield of marketable potatoes. This is considered to be of great importance in the eastern part of the United States where sulphur can be applied to the soil to bring about the desired acidity. Concerning the effect of increased alkalinity upon the occurrence of scab there is very little published evidence.

Most of the soils upon which potatoes are grown in western Nebraska are alkaline and the others are only slightly below the neutral point. The lowest pH value found in any of the soils tested in this survey was 5.92. All others were above 6.3 and 56 per cent were above 7.0 in the upper 6 inches. Table 3 shows the summarized data when the soils are grouped with each group having a range of 0.5 pH in the first 6 inches of soil. The figures for the second 6 inches were always slightly higher but still within the same pH group.

As might be expected in a survey of this type with so many uncontrolled factors, there was considerable variation in the fields in each group, as is shown in the probable errors. There was, however, a very evident and consistent decrease in both the amount and severity of scab as the pH values increased from 5.92 to 8.25. This result was exactly the opposite from what might be anticipated on the basis of the literature.

TABLE 3.—*The relation of the reaction of the soil to the occurrence of scab and the number of microorganisms*

Groups based on pH of first six inches	Scab record			Microorganisms per gram of soil							
	Number of fields	Total scab per cent	Medium and severe scab per cent	Number of fields	Total number of organisms (000 omitted)	Bacteria		Actinomycetes		Fungi	
						Number (000 omitted)	Per cent	Number (000 omitted)	Per cent	Number	Per cent
5.9-6.5	10	68.4 ± 5.72	16.4 ± 3.8	6	49,835	47,233	94.8	2,555 ± 121	5.2 ± 0.36	46,766	.09
6.5-7.0	29	56.8 ± 4.08	15.8 ± 2.7	13	37,254	34,750	93.3	2,465 ± 147	6.9 ± 0.33	39,033	.10
7.0-7.5	30	55.1 ± 3.30	15.5 ± 2.5	14	38,057	35,819	94.1	2,273 ± 160	6.2 ± 0.35	29,200	.07
7.5-8.0	13	48.0 ± 4.02	10.8 ± 1.8	9	47,400	45,340	95.6	2,031 ± 238	4.6 ± 0.54	29,465	.06
8.0-8.3	7	29.8 ± 5.49	7.9 ± 2.8	7	34,016	32,768	96.3	1,238 ± 92	4.1 ± 0.29	18,235	.05

As shown in table 3, the decrease in the amount of scab coincides with a decrease in both the total number and percentage of *Actinomyces*. There was no correlation between the total number of organisms and the occurrence of scab. There was no apparent relation between pH and the occurrence of *Fusarium* wilt.

*Soil Type.* The soils of 59 of the fields were classified in the Rosebud series and the remainder as Dunlap, Dawes, Valentine, Tripp, Epping, and Cheyenne. The number of fields in each of these latter were too small for purposes of series comparison. Twenty-eight of the fields were classified as silt loams and these averaged 67 per cent infection of scab. Twelve fields were loams and averaged 51 per cent infection, while 40 fields were very fine sandy loams and averaged 47 per cent infection. Samples plated from 12 silt loams, 9 loams, and 18 very fine sandy loams, chiefly from the Rosebud series, gave the following results in percentages of *Actinomyces*, 6.8, 5.0, 5.0, respectively. The total number of organisms was highest in the very fine sandy loams.

There was no correlation between the nitrogen content of the soil and either scab or wilt.

*Time of Planting.* Time of planting potatoes is known to be an important factor in influencing the development of scab in western Nebraska, late planted potatoes usually having the least scab due to the lower temperatures that prevail during tuber development. When the fields in this survey were grouped on the basis of date of planting, commencing June 1, the following average percentages of total scab were found for successive 10-day periods, 38.6, 51.9, 61.8, and 74.8 per cent. This reversal of the usual effect of late planting was probably due to the high rainfall during June and July, followed by dry weather in August and September, thus indicating that moisture may be more important than temperature in the development of scab. No correlation of date of planting and wilt was evident in these data, although observations and records over a period of years indicate that the same relationship holds as with scab.

*Weather Conditions.* There was no obvious correlation between either disease and temperature or rainfall as recorded by the U. S. Weather Bureau at 8 points in the area of the survey, or by the record of rainfall obtained by the use of rain gauges at 5 of the farms. The great variation in rainfall between fields only a few miles apart made it impossible to draw any conclusions from a comparison of large areas.

The rainfall at the 8 stations during June and July was above normal, the average rainfall being 3.71 and 3.45 inches, respectively. Only 2 stations reported less than 2 inches of rain in June and July. It was very dry during August and September, the average rainfall being 0.96 and 0.59 inches, respectively, with only 4 stations reporting over 1 inch in August and only 1 in September.

The mean monthly temperatures for 7 stations scattered through the survey area were as follows:—June, 59.0°; July, 70.9°; August, 70.1°; September, 59.7° F. The widest range occurring between these stations during any of the 4 months was 3 to 4° F. These temperatures were considerably below normal in June, slightly below in September, and about normal in July and August. In general, 1928, the year of this survey, was one of the worst scab years from 1925 to 1930.

*Soil Moisture.* The soil samples plated out varied from 8 to 32 per cent moisture. When all soils were grouped into 5 groups it was found that on a dry-weight basis the total number of organisms was higher in those few soils above 24 per cent. The percentage of bacteria was lowest and that of the Actinomyces was higher in the medium group, with the reverse occurring in the extremely dry or wet soils, while the percentage of fungi was higher in the wetter soils.

*Virgin Soils.* Comparative data from plate counts of virgin soils and adjoining cultivated soils were obtained from 9 fields and gave the following results:—average total organisms in virgin soils, 35,100,000 as against 31,900,000 for cultivated soils, bacteria 93.9 and 95.2 per cent, Actinomyces 6.2 and 4.8 per cent, and fungi .06 and .08 per cent, respectively. There was considerable variation, however, in the data from individual fields and the comparable virgin soils.

#### SUMMARY

A survey was made of the amount of infection of scab and *Fusarium* wilt from the soil in the high plains area of western Nebraska by planting portions of a lot of treated seed potatoes on each of 100 farms.

There were no fields entirely free from scab. The average percentage of infection of all fields was 10.5 per cent *slight*, 12.5 per cent *medium*, and 1.4 per cent *severe* scab with an additional 30 per cent of superficial scab.

Ninety-four per cent of the fields produced tubers with stem-end rot or vascular discoloration. The average for all fields was 4.5 per cent, with about one-half of this being of a severe type.

The following factors were correlated with a high percentage of scab infection: cultivated crops or summer fallow the preceding year, as contrasted with small grains, an interval of less than 4 years between potato crops, decreasing pH values of the soil from 8.25 to 5.92, silt-loam soils, as contrasted with loams or very fine sandy loams, and high number and percentages of Actinomyces in the soil.

No correlation was found in these fields between scab and content of organic matter, and rainfall or temperature, probably because of the greater influence of other limiting factors and the variability naturally occurring in a survey of this type.



There was no correlation between any of the factors recorded and the occurrence of *Fusarium* wilt as measured by stem-end rot or vascular discoloration except that less wilt occurred following legumes than after other crops.

The results here reported are considered of value chiefly in opening up further lines of inquiry, particularly as regards the effect of previous crops and of H-ion concentration of the soil, rather than as a practical recommendation for the control of these diseases. The results also clearly indicate that in the complicated field of soil-borne diseases conclusions drawn from observations or experiments in one section of the country do not necessarily hold for conditions in other sections.

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# AN UNDESCRIBED SCLEROTIUM FUNGUS PREVALENT IN NORTHEAST TEXAS

D. C. NEAL AND R. E. WESTER

(Accepted for publication August 11, 1933)

In September, 1932, following a period of wet weather, a fungus resembling *Phymatotrichum omnivorum* (Shear) Duggar was discovered at the United States Cotton Breeding Field Station, Greenville, Texas, growing on decayed cotton stalks and roots a few inches below the surface of the soil. It was first discovered in a field that had been subsoiled a few weeks earlier and brought to the attention of the writers by H. C. McNamara, Associate Agronomist and Superintendent of the Station. A few days later examinations of the various other plots revealed that it occurred not only on old fragments of cotton stalks and roots buried a few inches in the soil, but was rather abundant on the under surface of old cotton leaves which partly covered the soil. In many of the areas examined, the fungus had spread for some distance in the soil, forming abundant sclerotia as elongate swellings along mycelial strands (Fig. 1).

## DESCRIPTION OF MYCELIA AND SCLEROTIA

The mycelium of the fungus as found on the decayed roots and in the soil is white when young, but becomes pale yellow and occasionally ochraceous buff with age. It is uniformly septate, and branching is either opposite or alternate just below the septa (Fig. 2), with individual hypha averaging  $5.5\ \mu$  in diameter and  $60\ \mu$  in length. A characteristic behavior of new cells arising from the side of the primary hypha is to grow in opposite directions. In some cases, the lower portion of the cell grows downward for a short distance (see figure 2 at point indicated by arrow) and forms new cells either in parallel fashion or at an angle of  $45^\circ$  to the main filament. As a result of anastomosing of filaments the strand hyphae are formed, which later differentiate into sclerotia in the form of elongate swelling similar to the cotton-root-rot fungus.

The sclerotia are white to pale yellow, depending on age, and vary considerably in size and shape. As a rule, they average about 1 mm. in width and 5 mm. in length, being round to ovoid, ellipsoid, variously constricted, and often forked at the strand connections (Fig. 3). The exterior of the sclerotia is smooth or glabrous, and cross sections stained in crystal violet reveal cellular structures similar to those of cotton-root-rot sclerotia, composed of anastomosed hyaline hyphae.



FIG. 1. Sclerotia and strands of an undescribed sclerotium fungus observed on decayed cotton roots, stalks, and leaves in the soil at Greenville, Texas.  $\times 15$ .

#### CULTURAL STUDIES AND GROWTH ON MEDIA

The fungus is quite easily isolated and makes a rapid growth on a variety of culture media. One peculiar feature is that pure cultures are often obtained from sclerotia without subjecting the latter to previous surface sterilization with the usual toxic agents, such as mercuric chloride, alcohol, calcium hypochlorite, etc. On such media as carrot, corn-meal, and Dug-

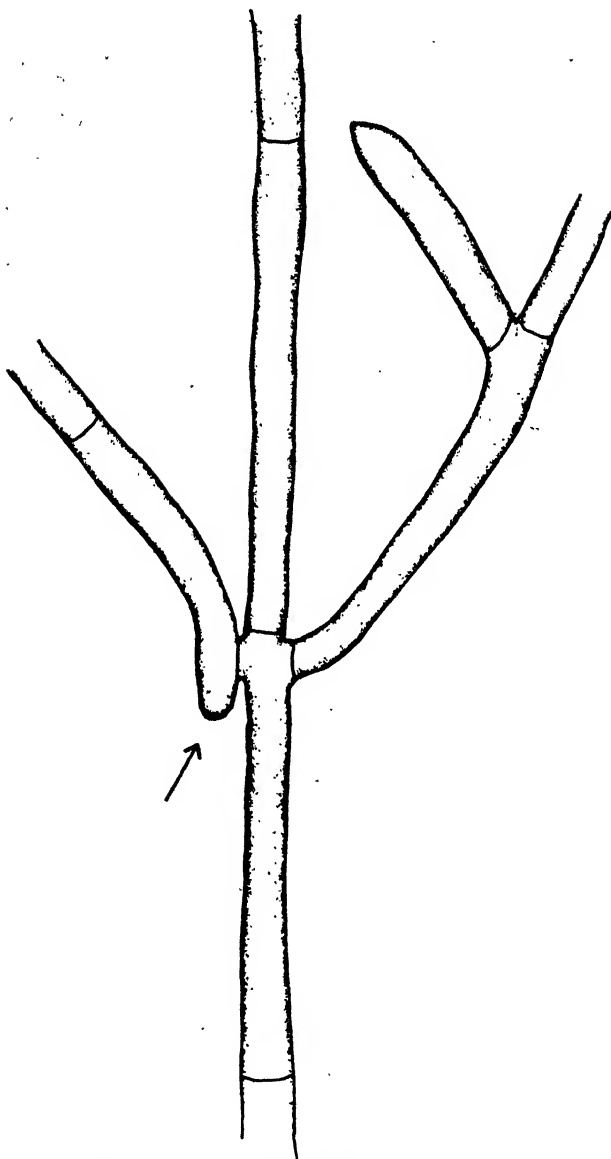


FIG. 2. Characteristic branching of the fungus after growing in a hanging-drop culture for 24 hours. Note septate mycelium and new hyphae arising just below the septa. Photographed from a camera-lucida drawing.  $\times 840$ .

gar's synthetic agar, sclerotia are formed in large numbers at a temperature of  $29^{\circ}$  C. In hanging-drop cultures, new filaments arise from the surface cells of the sclerotium and from those of the connecting strands within 24 hours (Fig. 4). These filaments anastomose freely, and rapidly give rise

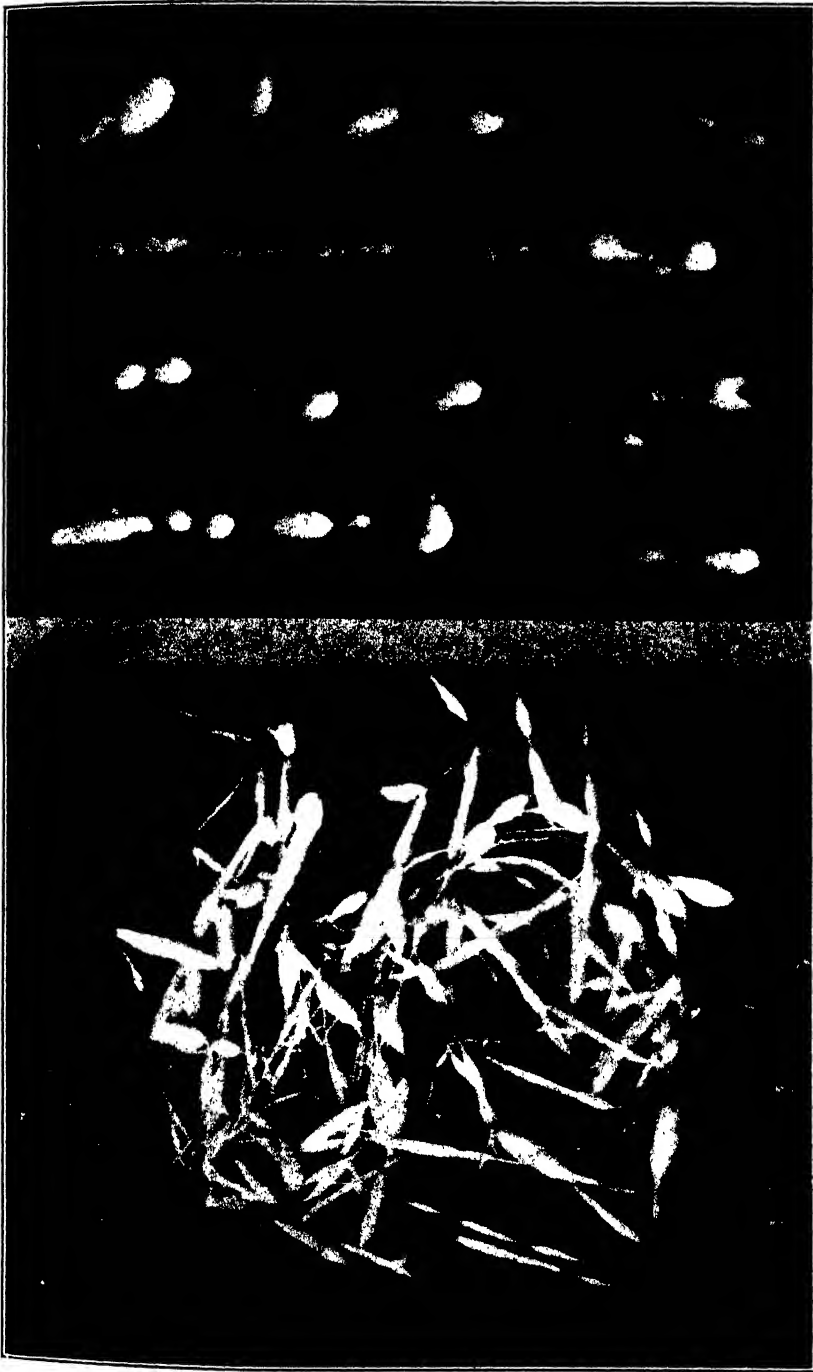


FIG. 3. Sclerotia and strands obtained from soil cultures of the fungus. A. Mass of sclerotia and strands from a soil culture 4½ months old. B. Sclerotia from a soil culture 8 days old. × 15.

to the arachnoid mycelium composing the strand. In quart-jar cultures of Wilson clay loam with a moisture content of 30 per cent and pH of 7 or 7.5, the strands ramify in the soil quickly and form sclerotia in large numbers within 3 or 4 days.

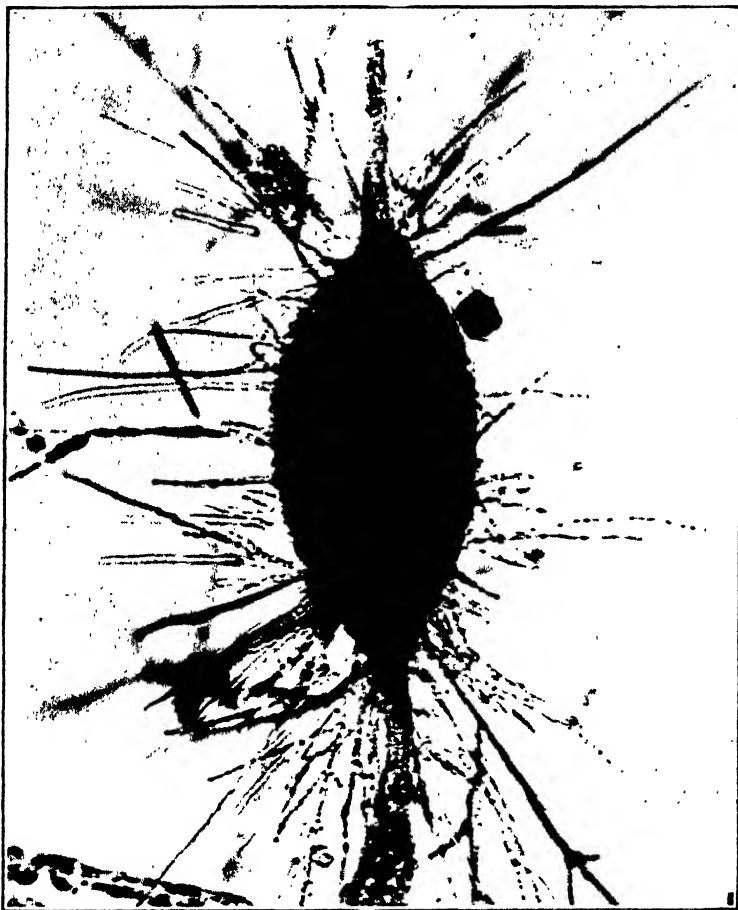


FIG. 4. A sclerotium with the connecting strand hyphae showing new filaments arising from the surface cells after being in a hanging-drop culture for 24 hours.  $\times 135$ .

Features that distinguish the fungus from *Phymatotrichum omnivorum* are the following: finer texture of the strands; rapid formation of sclerotia on agar; apparent saprophytic habits; and the ease with which the fungus is cultured. Also, in contrast to the root-rot fungus, the mycelium does not have right-angle branches or acicular hyphae. Since no spore stages were observed in any of the cultures previously mentioned, which varied in age from a few days to over 3 months, the writers believe that this probably is

a sterile fungus similar to the *Rhizoctonia*, *Sclerotium*, and *Ozonium* groups. In attempting to place it satisfactorily in any of the above genera, some difficulties have been encountered; however, the character of the hyphae and the presence of plectenchymatic strands preceding the development of sclerotia, appear to justify placing this fungus tentatively in the genus *Ozonium*. The species in question, insofar as the writers have been able to determine, has not been encountered or described heretofore by mycologists. Therefore, for taxonomic convenience in distinguishing this fungus from the parasitic *Ozonium* (*Phymatotrichum omnivorum*) responsible for cotton root rot and also widely prevalent in Texas, the name *Ozonium texanum*, sp. nov., is proposed.<sup>1</sup> A description of the organism is appended.

***Ozonium texanum*, sp. nov.**

Fructificatione ignota; mycelio sterile septato, juvenile et in culturis albedo, senescente pallide flavescence-ochraceo, infra, septa oppositer vel alterne ramoso; hyphis plerumque  $5.5\ \mu$  diam. et  $60\ \mu$  longis; in fasciculis plectenchymicis, elongata sclerotia formantibus, coalitis; hyphis novis saepe diversis vel ad angulos  $45^\circ$  ex filamentis crescentibus; sclerotiiis albidis vel pallide flavidis, in magnitudine et forma variabilibus, plerumque 1 mm. latis, 5 mm. longis, interdum rotundis vel ovoideis, generaliter ellipsoideis, radiceformibus, varie constrictis, saepe ad connectiones fasciculorum furcatis.

In radicibus, caulibus et foliis putridis *Gossypii* in terra, Greenville, Texas.

No fructification definitely known. Sterile mycelium septate, white when young or growing in cultures, becoming pale yellow to buff with age. Branching opposite or alternate below the septa, with hyphae averaging  $5.5\ \mu$  in diameter and  $60\ \mu$  in length. New hyphae frequently growing in opposite directions or at an angle of  $45^\circ$  to the primary filament. Hyphae forming plectenchymatic strands, which enlarge into elongate sclerotia. Sclerotia white to pale yellow, variable in size and shape, averaging 1 mm. in width to 5 mm. in length, occasionally round to ovoid, but usually ellipsoid, root-like, variously constricted, and often forked at strand connections.

Hab. Hyphae and sclerotia on decayed cotton roots, stalks, and leaves in the soil at Greenville, Texas.

Cultures have been deposited in the Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

BUREAU OF PLANT INDUSTRY,

U. S. DEPARTMENT OF AGRICULTURE,

GREENVILLE, TEXAS.

<sup>1</sup> Neal, D. C., and R. E. Wester. An undescribed sclerotium fungus prevalent in northeast Texas. *Phytopath.* 23: 24. 1933.

# A BACTERIAL CANKER OF PEAR TREES NEW TO CALIFORNIA

EDWARD E. WILSON

(Accepted for publication July 12, 1933)

In the autumn of 1931 a cankerous disease of Wilder pear branches was found in an orchard near Penryn, Placer County, California. A preliminary survey showed that the same type of canker was present on trees of the Beurre Bosc and Comet varieties; the Bartlett variety, on the other hand, being relatively free of the disease. Similar cankers have since been found in El Dorado, Sacramento, Lake, and Mendocino Counties on Easter Beurre, Winter Nellis and Beurre Hardy varieties of pears, and on an unknown variety of apple.

The external appearance of newly formed cankers is rather characteristic in that the periderm becomes loosened and raised (Fig. 1, D), portions of it sloughing away to expose the spongy, disorganized cortex beneath. In active cankers, the cortex is light tan or buff; brownish streaks may extend upward and downward through the cortex and outer phloem for a distance of several inches beyond the outwardly visible canker. During the summer, healing beneath the diseased area causes the outer bark to become longitudinally and transversely cracked (Fig. 1, A, B). At the same time the streaks at the apical margins of the cankers change from a light brown to a dark brown or almost black color (Fig. 1, C). Another characteristic feature of the cankers is their confinement to the cortex and outer phloem. Thus, while the disease may eventually kill limbs or entire trees, it does so rather slowly in average years. In the spring of 1933, on the other hand, a number of pear trees were killed in Placer County. Some of these had been diseased for several years, others had only recently become so.

Field diagnosis of this canker may be difficult in orchards where fire blight is present and it seems probable that these diseases have often been confused. It is desirable, therefore, to point out some of their contrasting symptoms.

*Period of Activity of Disease.* The new disease is active in the cool weather of late autumn, winter, and early spring. Fire blight is most active in the warm weather of late spring and summer.

*Tissues Involved by the Disease.* The disease is primarily one of the outer bark, although it may in certain years, such as 1933, involve all of the bark. Fire blight, although first involving the cortex, eventually extends to the cambium.

*External Appearance of Cankers.* The cankers of the new disease are first visible as light brown irregular patches on the limbs, the brownish



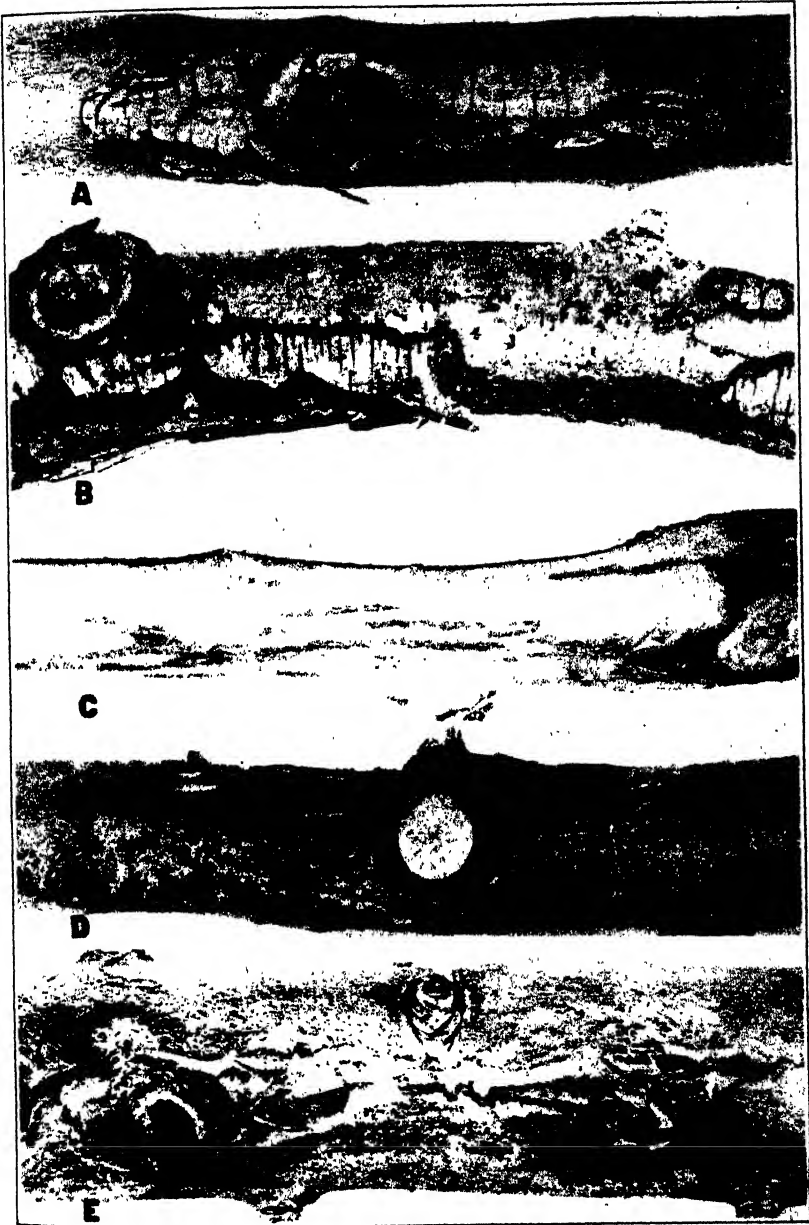


FIG. 1. A-D. Cankers from natural infection. A. Canker surrounding a lateral spur. B. Longitudinal and transverse cracking of bark over diseased areas. C. Black streaks extending through the outer bark of an infected branch (periderm removed). D. Active canker originating at a pruning wound. Periderm is loose and beginning to slough away. E. Symptoms produced by inoculating a Wilder pear branch.

color being due to the loosened periderm. Later the periderm may wholly or partially slough away, exposing the spongy cortex. Cankers of fire blight, on the other hand, are dark grey to black and the periderm usually remains firmly attached.

*Internal Appearance of Cankers.* Bark affected by the new disease is spongy and of a tan or buff color. Numerous fine brown streaks often extend from the apical margins of affected areas into otherwise apparently healthy bark. In fire-blight cankers the diseased tissue is first water-soaked; later, a distinct reddish-brown color appears. The cortex remains firm; streaks, if present, are few in number.

Isolations from the cankers of the cortical disease yielded bacteria consistently. In comparatively inactive cankers the best results were obtained by removing material for isolation from the zone of streaks at the lower or upper canker margins. The organisms from different sources, although differing in chromogenic characters on potato-dextrose agar, were on the whole similar.

Inoculation experiments, conducted during the winters of 1931-32 and 1932-33, showed that the bacteria were capable of producing symptoms on pear branches in every way similar to those in nature (Fig. 1, E). Organisms similar to those used in the inoculation work were obtained upon re-isolation, and these bacteria in turn produced cankers when inoculated into pear branches. In order to guard against the inclusion of *Bacillus amylovorus* in the inoculum or its entry into the wounds after inoculation, the cultures were punctured into green pear fruits. The bacteria obtained in the reisolations from limb cankers were also inoculated into green pears. In no case did the characteristic fire-blight symptoms develop on the fruit. An additional guard against infection by *B. amylovorus* was afforded by the numerous control inoculations. In only 3 cases out of several hundred did the control punctures develop symptoms; these being those of the new disease.

A description of the causal organism is not given at this time, inasmuch as cultural studies are not complete and comparisons with certain other organisms are desirable. The bacterium differs from *Bacillus amylovorus*, both in type of symptoms produced on the host and in cultural characters. For example, it produces a greenish pigment on many media, while *B. amylovorus* produces no pigment. In this feature it allies itself with certain earlier named organisms. For example, the inoculation work indicated that it is similar to if not identical with the organism, *Pseudomonas cerasi*, producing cankers in stone-fruit trees. The writer has earlier

shown (4) that the stone-fruit organism<sup>1</sup> is very similar to *Pseudomonas prunicola*, reported by Wormald (7) as the cause of a-green-shoot blight of plums in England. An organism described as *Phytomonas utiformica* by Clara (1) in New York is being compared with the California pear organism. Thus far the 2 organisms have proved similar.

Rosen (2) in 1932 mentioned a bacterial blossom and twig blight of pears and apples, the organism of which was similar to *Pseudomonas prunicola*. Later, (3) he made pathological and serological comparisons of a number of organisms, including his pear pathogen, *P. prunicola*, *Bacterium syringae*, and *B. citriputeale*, and found them to be indistinguishable.

A blossom blight of pears, differing from that produced by *Bacillus amylovorus*, has been found in California by Harvey E. Thomas. The organism obtained from such symptoms appears similar to those found in the cankers, described herein. Thus far, however, no detailed pathogenicity comparisons have been made of bacteria from the 2 sources. Such comparisons are planned and will be made the subject of a paper by Dr. Thomas.

Obviously, the organism mentioned herein cannot be assigned a name until it is compared with other similar organisms. It is becoming more and more evident that new species created from studies of the disease of a single host are often unjustified.

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<sup>1</sup> In his earlier publication (4) the writer stated that two chromogenic types of the stone-fruit organism existed. Later (5) he reported that one of these was considered to be *Pseudomonas cerasi*, the other a variety of the same species. A more recent article (6) deals with these types or varieties of *P. cerasi*.

# STEM CANKER OF HOLLYHOCK CAUSED BY SCLEROTINIA SCLEROTIORUM<sup>1</sup>

PAUL A. YOUNG

(Accepted for publication August 7, 1933)

White and retted cankers were found in many stems of hollyhock, *Althaea rosea* Cav., in a garden in Bozeman, Montana, on October 17, 1931. This stem canker was studied during 1931 to 1933 because it resembled sunflower wilt, and because the hollyhock is a valuable ornamental plant.

## REVIEW OF LITERATURE

Smith<sup>2</sup> described the stem canker of hollyhock. He published drawings of sclerotia in stems, and of sclerotia bearing immature and mature apothecia, named *Peziza sclerotiorum*. Richardson<sup>3</sup> described the stem canker of hollyhock and reported successful inoculations with a species of *Sclerotinia*. Young<sup>4</sup> described the *Sclerotinia* stem canker of hollyhock in Montana. Young and Morris<sup>5</sup> described the similar symptoms of *Sclerotinia* wilt of sunflower.

## EXPERIMENTAL RESULTS

*Symptoms and Signs in Hollyhock.* Black to dark green, water-soaked, rapidly enlarging cankers appeared in the cortical tissues near the wounds in artificially inoculated hollyhock stems. The centers of the cankers dried and became white, with black or dark green margins. Most of the cankers girdled the stems within 2 to 4 weeks. Later, the leaves wilted on the stems above the cankers.

Mature cankers from natural and artificial inoculations were 5 to 45 cm. long, and had black, brown, or indefinite margins. Black and brown streaks appeared in the epidermis and xylem from 1 to 15 cm. above some cankers. Brown cankers developed on some tap roots attached to stems with basal cankers.

The epidermis of mature cankers was white or light brown (Fig. 1, A). Many cankers had 2 to many, nearly concentric, brown arc lines with di-

<sup>1</sup> Contribution from Montana State College, Agricultural Experiment Station, Paper 37, Journal Series. The writer wishes to thank Professor H. E. Morris for valuable suggestions.

<sup>2</sup> Smith, W. G. New disease of hollyhocks. Gard. Chron. III, 8: 324. 1890; and III, 9: 791-792. 1891.

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<sup>4</sup> Young, Paul A. *Sclerotinia* stem canker of hollyhock. Phytopath. 23: 39. 1933.

<sup>5</sup> Young, P. A., and H. E. Morris. *Sclerotinia* wilt of sunflowers. Mont. Agr. Exp. Sta. Bul. 208. 1927.



FIG. 1. Hollyhock stems naturally infected with *Sclerotinia sclerotiorum*. A. Canker with white, cracked epidermis and retted, protruding bast fibers. A sclerotium is shown.  $\times 1$ . B. Canker with white epidermis and black sclerotia.  $\times 2$ . C. Hollowed stem showing mycelium.  $\times 1.3$ .

mensions of 3 to 15 by 0.3 to 1 mm. Some of these are lines marked the tops of epidermal wrinkles.

Hyphae grew below and in the epidermis, and emerged as white, conical pimples 0.3 to 1 mm. wide in the epidermis (Fig. 1, B). The pimples consisted of hyphae and epidermal cells. Large masses of hyphae emerged through the epidermis and formed black sclerotia (Fig. 1, B).

The dried epidermis remained intact on the cankers during a month or longer. Later, the epidermis on many cankers ruptured and released the retted bast fibers, which protruded from the cankers (Fig. 1, A, C).

This fungus decomposed the pith in the cankered parts of stems and formed dense masses of mycelium and sclerotia in the cavities thus formed (Fig. 1, C). It caused chambered pith in some hollyhock stems, resembling the chambered pith of *Juglans nigra* L.

The cankers caused by *Sclerotinia sclerotiorum* in hollyhock closely resembled the cankers caused by this fungus in sunflower.

Thick layers of white hyphae with sclerotia developed on the bases of many cankered, dead hollyhock stems in the garden during February to April, 1932. *Sclerotinia sclerotiorum* was isolated from one of these stems, and was used in the inoculations.

*Inoculation of Hollyhocks.* Two hundred hollyhocks, 1 to 3 years old, were transplanted on April 11, 1932, to a field where they grew normally. Blocks of agar bearing the *Sclerotinia sclerotiorum* isolated from hollyhock were placed in wounds in many of these living hollyhock stems and the wounds were covered with wet cotton. These stems were inoculated on July 16, 1932, and Sept. 1, 1932.

Cankers developed in 71 of these 75 inoculated stems within 13 days and killed the stems within 30 to 60 days. These cankers were essentially identical with the cankers from natural infections in the garden.

*Evidence of Resistance.* Cankers appeared only in the artificially inoculated hollyhocks in this field. The roots of these cankered plants apparently were not attacked by the fungus within 4 months. Evidently, hollyhocks are more resistant to *Sclerotinia sclerotiorum* than are sunflowers, because this fungus usually kills sunflower roots and commonly spreads to neighboring plants.

*Inoculation of Sunflower (*Helianthus annuus* L. var. White Beauty).* Blocks of agar bearing the *Sclerotinia* isolated from hollyhock were placed in wounds in large, living sunflower stems, and the wounds were covered with wet cotton. These inoculations were made on July 16, 1932, and Sept. 1, 1932. Cankers developed in 18 of the 21 inoculated stems within 5 to 13 days, and quickly rotted the cortical parenchyma tissues. The *Sclerotinia* spread into the roots of the infected stems and killed the plants within 60 days.

An inoculated sunflower head developed a canker that rotted the parenchyma tissues in 2/3 of the head within 13 days. This canker bore 4 large sclerotia in the spongy part and many thin flat sclerotia in the seed layer within 39 days.

These cankers in the stems and head were like those described by Young and Morris.<sup>6</sup>

*Reisolations and Cultural Characters of Sclerotinia sclerotiorum.* Isolations from 3 cankers in hollyhock stems and from 1 canker in a sunflower stem all gave pure cultures of a *Sclerotinia* identical with the cultures used in inoculating these stems. All of these cultures produced abundant white mycelium fuzzy white immature sclerotia bearing hyaline droplets of liquid, and numerous mature black sclerotia with dimensions of 1 to 10 by 1 to 5 mm. These characters are typical of *Sclerotinia sclerotiorum*.

*Germination of the Sclerotia.* Sclerotia and mycelium with attached pieces of agar were cut from pure cultures of *Sclerotinia sclerotiorum* and were planted in the soil in 6 flower pots in the greenhouse. These agar cultures were transfers of the cultures used in inoculating the hollyhocks and sunflowers. The sclerotia grown on agar produced 11 mature apothecia and 3 filiform bodies within 94 to 173 days in 4 of the pots.

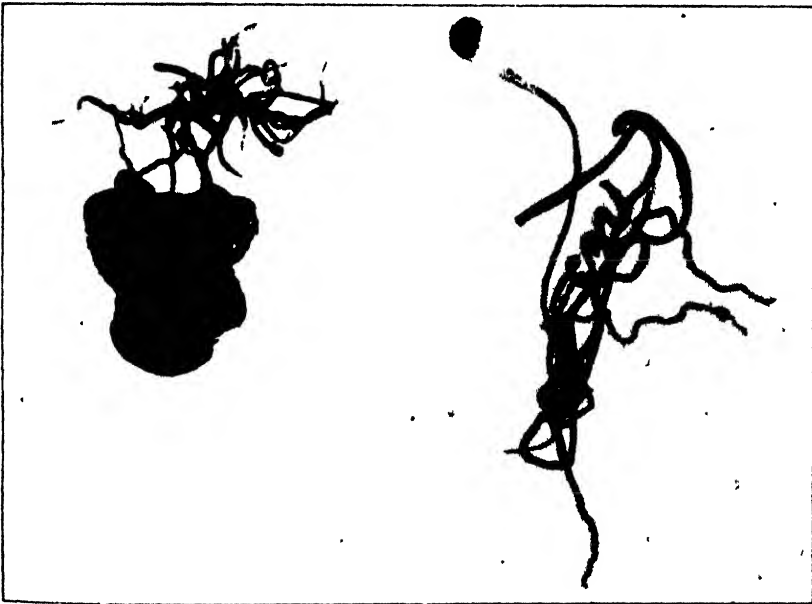


FIG. 2. Filiform bodies and a clavate, atypical apothecium of *Sclerotinia sclerotiorum* produced within 52 days by sclerotia from a sunflower root.  $\times 1.8$ .

<sup>6</sup> See footnote 5.

Sclerotia from the cankers in the stems of hollyhocks and sunflowers were planted in the soil in flower pots. These cankers were caused by *Sclerotinia sclerotiorum* in the artificial inoculations. The sclerotia grown inside hollyhock stems produced 4 mature apothecia and 1 filiform body within 115 to 141 days in 3 of the 28 pots. The sclerotia grown on sunflower roots and inside sunflower stems produced 2 mature apothecia and 15 filiform bodies (Fig. 2) within 52 to 100 days in 4 of the 9 pots. Mature apothecia were illustrated by Young and Morris,<sup>7</sup> who described the method used in germinating sclerotia.

The apothecia were 1 to 5 mm. wide and avellaneous brown. Their stipes were 5 to 20 mm. long, with black to dark brown lower parts and light brown upper parts.

The filiform bodies (Fig. 2) were immature apothecia, and were black to dark brown, like the bases of apothecial stipes. Some of the filiform bodies produced mature apothecia, but others produced only light brown tips that did not become apothecia. Many of these filiform bodies remained unchanged in shape and color and did not form apothecia.

One of the sclerotia grown on agar produced an apothecium 5 mm. in diameter. Fragments of it studied in distilled water revealed asci 108 to 144 by 10 microns, with 8 spores per ascus; ascospores 9 to 12 by 5 to 7 microns; and clavulate paraphyses 81 to 100 by 3 microns. Iodine in an aqueous solution of potassium iodide made the tips of the asci blue. Essentially identical asci and spores were found in the similar apothecia produced by sclerotia grown inside cankers in hollyhock stems.

*Name of the Fungus Causing Stem Canker of Hollyhock.* The morphological characters of the fungus causing stem canker of hollyhock all agree with the description of *Sclerotinia sclerotiorum* (Lib.) deBary.

#### SUMMARY

*Sclerotinia* stem-cankers in hollyhocks exhibited the following symptoms and signs: white or light brown cankers 5 to 45 cm. long and girdling the stems; sclerotia, white pimples, and brown arc-lines on many cankers; retted bast fibers projecting from cankers; and sclerotia in stems made hollow by the fungus.

The fungus isolated from hollyhock was inoculated into hollyhock stems in which it produced symptoms and signs essentially like those resulting from natural infections. This fungus also was inoculated into sunflower stems in which it produced symptoms and signs identical with those of *Sclerotinia* wilt of sunflower. The fungus reisolated from these cankers in

<sup>7</sup> See footnote 5.



hollyhock and sunflower stems was identical with the fungus used in inoculating these stems.

*Sclerotia* grown in pure cultures on agar produced apothecia and filiform bodies. *Sclerotia* grown in cankers in hollyhock and sunflower stems also produced apothecia and filiform bodies. The filiform bodies were immature apothecia.

Morphological characters showed that the fungus causing stem canker of hollyhock is *Sclerotinia sclerotiorum* (Lib.) deBary.

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# ABNORMAL GERMINATION IN DUSTED WHEAT<sup>1</sup>

WILLARD CROSIER

(Accepted for publication July 14, 1933)

The injury to cereals occasioned through treatment with various dips, formaldehyde in particular, has led gradually to their abandonment in favor of the fungicidal dusts. In general the dust treatments, if made with reasonable care, have not resulted in injury, and reports of increased field stands, which may indicate increased percentages of germination, are not uncommon. Lambert *et al.*<sup>2</sup> determined the efficiency of various dusts and liquids in controlling the covered smuts of small grains. They found no indication of germination abnormalities with any dust, although applications of formaldehyde caused marked seed injury. Briggs<sup>3</sup> experimented with 24 commercially compounded dusts and copper carbonate, applying them at rates of 1 to 4 ounces per bushel. He states that no seed injury was encountered and that several cases of increased field stands were noticed.

In July of 1932 a sample of Marquis spring wheat was received by the Seed Laboratory for germination tests. The gray color of the seed, as a whole, as well as dust deposits on the brush end, were evidences that the sample had been drawn from dusted seed. Triplicate sets of 100 seeds each were placed to germinate at 18–20° C. Counts made after a 5-day germination period showed that the seedlings were abnormal in respect to strength and type of sprouts (Fig. 1). The average percentages were: normal seedlings 49; nearly normal plumules but short thickened roots 13; very abnormal plumules and roots 34 and dead seeds 4. With several seedlings the roots were clubbed and thickened to about 4 times the normal diameter and were only a fraction of the normal length. The germination tests were continued for 10 days longer in the expectation that the deformed seedlings would become more nearly normal. However, only 6 of the slightly abnormal seedlings developed into normal ones. From all indications this sample would not produce a satisfactory field stand. This was confirmed later by the vendor who stated that poor stands occurred in Maine and that the Experiment Station at Orono reported an unsatisfactory field stand in its plots.

<sup>1</sup> Published as Journal Paper No. 1 with the approval of the Director of the New York State Agricultural Experiment Station.

<sup>2</sup> Lambert, E. B., H. A. Rodenhiser and H. H. Flor. The effectiveness of various fungicides in controlling the covered smuts of small grains. *Phytopath.* 16: 393–412. 1926.

<sup>3</sup> Briggs, Fred N. Seed treatments for the control of bunt of wheat. *Phytopath.* 16: 829–842. 1926.

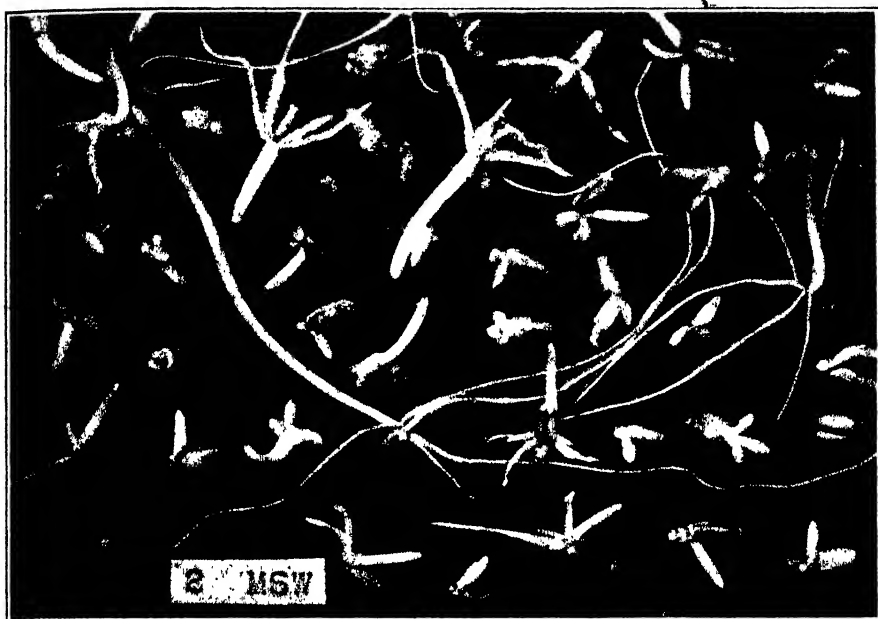


FIG. 1. Seedlings of Ceresan-dusted Marquis spring wheat after a germination period of 5 days.

The seed had been treated with Ceresan in March, 1931, was thoroughly dry at the time of the dust application, and had been held in cool, dry storage until the spring of 1932. No record of the field performance in 1931 was obtained. However, the vendor concluded from germination tests that the seed would produce normal field stands. Other seed lots, similarly treated and stored, were normal in both laboratory tests and field performance.

Additional laboratory germination tests were performed in November, 1932, or about 4 months after the first tests. In 10 lots of 100 seeds each, subjected to slightly different temperature and moisture relations, the percentages of normal germination varied from 54 to 33, the average of the entire 1000 seeds being 49. Washing the seeds in distilled water, brushing to remove the adhering dust deposits, or soaking in well-aerated running water for several hours had no effect on the type or rapidity of germination. The sample was tested the third time in February. The germination was read in 6 and in 16 days. In every lot a few seedlings placed in the "Nearly normal plumules but abnormal roots" list at 6 days were advanced to the "Normal" list at 16 days. None of the decidedly abnormal seedlings improved during this period. A comparison of these germination percentages appears in table 1.

TABLE 1.—*Percentages of germination in Marquis spring wheat dusted with Ceresan*

Date of test	Length of test in days	Condition of seedlings			
		Normal	Abnormal roots and nearly normal plumules	Abnormal plumules and roots	Dead seeds
1932 .....	Growing season		Very unsatisfactory field stand		
July, 1932 .....	5	49	13	34	4
July, 1932 .....	15	55	7	34	4
November, 1932	5	49	14	30	7
February, 1933	6	52	16	26	6
February, 1933	16	60	8	26	6

The vendor's declaration that the orthodox method of treatment had been followed led naturally to the probability of other causes for this abnormal germination. Several hundred seeds were examined minutely under a binocular microscope. The seed coats were cracked or chipped in some grains so that the embryo was poorly protected. The percentages of injured and noninjured seeds were: badly cracked coats 4.6, chipped coats 46.4, and coats intact 49. It is not impossible that such a condition arose from vigorous mixing of the seed during treatment. A comparison of the germination of the seeds so classified is made in table 2.

TABLE 2.—*Percentages of germination in injured and noninjured wheat seed dusted with Ceresan*

Condition of seed coats	Variety	Normal sprouts	Abnormal roots nearly normal plumules	Very abnormal roots and plumules	Dead seeds
Cracked	Marquis spring wheat	9	17	71	3
Chipped		21	5	67	7
Intact		69	15	13	3
Chipped	Honor winter wheat	61	10	5	24
Intact		99	0	0	1
Bulk		96	1	0	3

Unfortunately, this entire lot of wheat had been treated so it was impossible to ascertain whether the seed-coat injury should be attributed to the mechanics associated with the dusting or to other causes. This also made it impossible to compare the germination of the dusted wheat with that of nondusted wheat of the same lot.

To see if this condition was at all common a sample of farmer-treated

(copper carbonate) seed was examined and placed to germinate. This sample had been stored for over a year in a warm laboratory. Of 1000 seeds examined, cracking or chipping was found in 81. Germination results are recorded in table 2. The abnormal seedlings were closely comparable to those observed in the sample of Marquis. Chipped seed coats also were found in several nontreated lots of wheat. However, even after a year's storage, there was no appreciable difference in the germination of the seeds with chipped coats and with coats intact.

Attempts to induce abnormal germination by chipping the seed coats and dusting with Ceresan were unsuccessful. Seeds that had been severely bruised, chipped, or cracked and then liberally dusted were stored in tight containers for 3 months. No diminution in germination was observed. On the other hand, seeds treated while moist germinated very poorly after 3 months' storage. In most cases very few sprouts could be found.

It would seem that the question of seed treatment is in need of further consideration. Some attention also might be directed justifiably toward safe methods of treatment and subsequent storage for large-quantity trade lots of cereals.

#### SUMMARY

Abnormal laboratory germination has been observed in a sample of dusted Marquis wheat. It is thought that the dust injury to the seed was accentuated either by chipping of the seed coats or by faulty storage conditions.

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# VARIABILITY OF *PSEUDOMONAS CERASI* IN PHYSICAL CHARACTERISTICS OF GROWTH ON SOLID MEDIA

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(Accepted for publication July 12, 1933)

The shape, size, color, elevation, and consistency of the colony have long been considered important features in identifying bacteria. That is to say, a bacterial colony is reported to be flat or raised, of a butyrous, slimy, or gelatinous consistency and of a white or yellow color. The investigator may, early in the work, conclude that certain of these features are constant for his organism. In isolations from diseased tissue, he will select those colonies that conform to these characters and discard all others. This practice delays the recognition of variations within the same species, unless such variations are frequent in pure culture.

Studies of a canker of stone-fruit trees in California have revealed that not only does the pathogen, *Pseudomonas cerasi* Griffin, vary in pigment production,<sup>1</sup> but more recently the organism has been found to undergo, at times, a change in physical characteristics of the colony. Isolations from the cankers have occasionally yielded colonies very different from the more common type in elevation, topography, and consistency, whereas the colonies of the common type on potato-dextrose agar are rather flat, thin-margined, smooth-surfaced and of a butyrous consistency (Fig. 1, A); those of the variant are raised, with convolute surfaces and of a gelatinous consistency. Transfers of such colonies to potato dextrose agar slants resulted in a raised, convolute, gelatinous growth (Fig. 1, C). This type of growth persisted in certain cases for several months, although it was noted that in all cases the gelatinous character would eventually be lost in subsequent transfers and the flat, smooth-surfaced, butyrous growth appear instead.

Had not the colonies of the gelatinous type produced pigment similar to that formed by the typical *Pseudomonas cerasi*, they probably would have been regarded as those of an unrelated organism. Later evidence strengthened the conviction that the *Ps. cerasi* actually produced 2 distinct colony types. A culture of *Ps. cerasi* that had been subjected to the usual purification procedure a number of times was seen to change from the flat, butyrous condition into the raised, gelatinous condition. The first indication of such a change was the appearance of small, hemispherical pustules at dif-

<sup>1</sup> The existence of two chromogenic types of bacteria capable of producing identical symptoms was reported (5) in 1931. At the Atlantic City meetings the writer (6) stated that one of these types is considered to be *Pseudomonas cerasi*; the other a strain of the same species. Recently (7) these two strains have been described in more detail.

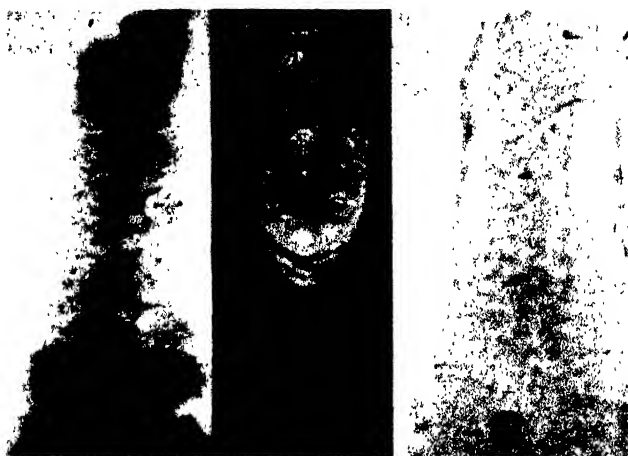


FIG. 1. Variation of *Ps. cerasi* in culture. A. Typical flat, thin-edged growth of butyrous consistency. Photographed by transmitted light. B. Pustules beginning to appear at different places in a typical flat streak of the organism. C. Raised, spongy, gelatinous growth produced by bacteria transferred from pustules of B. Photographed by transmitted light.

ferent places in the streak (Fig. 1, B). These pustules enlarged until the entire surface of the culture was covered by a gelatinous growth with a convolute surface. By plating out the bacteria from both types of growth in the same culture, it was found, in a limited number of trials, that the bacteria from the pustules produced only gelatinous colonies, while those from the flat areas produced both gelatinous and butyrous colonies.

Recently a single-cell culture of *Pseudomonas cerasi* changed from the butyrous into the gelatinous type. It has been possible to make a limited study of this variant. Earlier attempts at comparisons of the two types had resulted in inconclusive results, due to the reversion of the variant to the parent type before the studies were completed. In the case of the single-cell culture, however, the variant was maintained in culture long enough to conduct a number of inoculation and cultural experiments. These experiments showed that the variant was pathogenic, although it did not produce as extensive cankers as did the typical *Ps. cerasi*. The cultural features, as far as studied, were practically the same for both types. The variant did not appear to produce as much acid on sucrose and glycerin as did the parent type, but on 18 other carbon compounds no consistent differences could be found.

Although this phenomenon has not been studied sufficiently to warrant a conclusion as to its nature, a comparison of figure 1, B with photographs published by Hadley (4, plate 1) will show a striking resemblance between the pustules produced by *Pseudomonas cerasi* and secondary or daughter

colonies formed by *Vibrio cholerae* and *Bacillus typhosus*. Hadley (4, page 58) has the following to say concerning daughter colonies: "We have noted in many instances that the ability of a colony to generate papillae or daughter colonies seems invariably to be correlated with reactions of dissociative significance." Whether the variation reported herein is a manifestation of dissociation remains to be proved.

The occurrence of the variation affords a vein for speculation on the possible relationship between *Pseudomonas cerasi* and an earlier described canker-producing organism, *Ps. spongiosa* Aderhold and Ruhland (1, 2). In describing *Ps. cerasi*, Griffin (3) called attention to Aderhold and Ruhland's organism, and, although he considered the two similar, stated that he had never observed *Ps. cerasi* producing the "spongy" colonies attributed to *Ps. spongiosa*. Aderhold and Ruhland may have had reference to the gelatinous, convolute-colony characters similar to those mentioned herein. To say that nothing further stands in the way of considering the 2 organisms identical, would be unwarranted, since their descriptions differ in other details. *Ps. spongiosa* will remain in the literature as an inadequately described species until these differences can be harmonized or the organism is shown to be a distinct species.

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## PHYTOPATHOLOGICAL NOTES

*Further Observations on the Natural Distribution of the Cotton Root-rot Fungus.*—The natural distribution of the fungus (*Phymatotrichum omnivorum* (Shear) Duggar), the cause of a cotton root rot, extends to the desert regions of the Southwestern States, as indicated in the report of an occurrence of the fungus on the roots of several species of desert plants near Florence, Arizona.<sup>1</sup> Later observations have extended to several other districts, and the fungus has been found on the roots of two species of palo-verde and other prominent desert plants.

It now appears probable that the root-rot fungus will be found indigenous to most of the desert region included in the drainage basins of the Rio Grande, Gila, and Colorado Rivers.

The disease has been recognized recently in southern Utah, which may be considered provisionally as the northern limit of the fungus, while on the south it extends into Sonora and Lower California.

The *Phymatotrichum omnivorum* was found in Oct., 1933, on dead and dying plants of the Mexican poppy *Argemone* sp., a short distance south of the international boundary in a mountain wash that drains through the settlement at Jacumba, Calif., where an infestation had been found in some alfalfa fields in Sept., 1932.

The conditions of natural occurrence in the desert differ widely from those in the cultivated fields, where the ravages of the disease have attracted attention. Under the usual dry conditions the fungus does not appear on the surface, and the scattered desert plants may not be killed by the disease, even where many of the roots are found to be affected.

Only by the chance find of a dead plant, or by the spore mats coming to the surface in wet weather, can the fungus be detected. In the Gila Valley the infestations in the earliest susceptible crops often are traced to infected mesquite roots or stumps or the shrub *Lycium*, commonly known as "squawberry." In some cases, however, other native forms apparently are "carriers" of infection to cultivated plants. In addition to the native species listed in the previous paper<sup>1</sup> as root-rot carriers, the mycelium of the fungus was found, in 1933, on the roots of *Franseria confertiflora*, *Gutierrezia lucida*, *Platanus wrightii*, *Parkinsonia aculeata*, *Aster spinosa*, and *Cercidium torreyanum*.

Discovery of infestations on native plants in these widely separated areas makes it apparent that the fungus has there a natural distribution. Little is now known of the factors that influence its distribution. Recent

<sup>1</sup> Science, January 8, 1932, page 48.

evidence obtained by the writers indicates that the movement of water in erosion and drainage may play an important part. Infestations in the cultivated areas of Arizona and southern California are located chiefly along the flood plains of the principal irrigation streams. It is probable that some of these may have originated from the lodgment of infected driftwood on the bottomlands during the flood season or in the distribution of irrigation water.

In some of the irrigation projects the lands nearest the rivers and those with the oldest riparian rights show more extensive infestations than the lands to which the water was only recently applied. Infestations have been found in the watershed areas of some of the larger projects, and in one case infected native plants and spore mats were found in several places on the steep hills surrounding the principal irrigation reservoir and many miles upstream from the nearest irrigated fields.

In 1933 the writers observed an extraordinary behavior of root rot in some cotton plantings on newly cleared land near Coolidge, Arizona. The area, before clearing, was of the mesa type made up of a highly calcareous soil with outcroppings of "caliche," and was covered largely with the creosote bush *Covillea tridentata*. The area was traversed by 3 or 4 washes that drained northwestward toward the Gila River. In leveling the land for crops these washes were filled in places where they were shallow, but were largely left open and cotton was planted as close to their banks as possible. In Oct., 1933, it was noted that areas of dead cotton plants lay in long narrow strips along the banks of the washes. In some cases these irregular strips were continuous for more than  $\frac{1}{2}$  mile (Fig. 1), and the tendency of paralleling the stream beds was noted for nearly  $1\frac{1}{2}$  miles.

Root-rot infestations can be found to some extent in nearly all of the tilled areas along the Gila River throughout the 350 miles of its course, from western New Mexico to its junction with the Colorado River at Yuma, Arizona. Infestations along the Colorado River system are known as far north as Washington County, Utah, and are extensive in some of the irrigated areas near Blythe, Calif., and Parker and Yuma, Arizona.

In reporting the discovery of the infestation in southern Utah (Plant Disease Reporter, May 15, 1933) B. L. Richards of the Utah Agricultural College stated as follows:

"A survey in Washington County, Utah, discovered for us the presence of *Phymatotrichum* root-rot in this particular locality. At present the disease is spread generally throughout the townships of Ivins, Santa Clara, St. George, and Hurricane, and is doing tremendous damage to the various crops. It is evident from our survey that the organism has been in the county probably since settlement by the pioneers and is probably indigenous to the region, as a number of fields exhibited root-rot disease the first year after being reclaimed from the native vegetation."

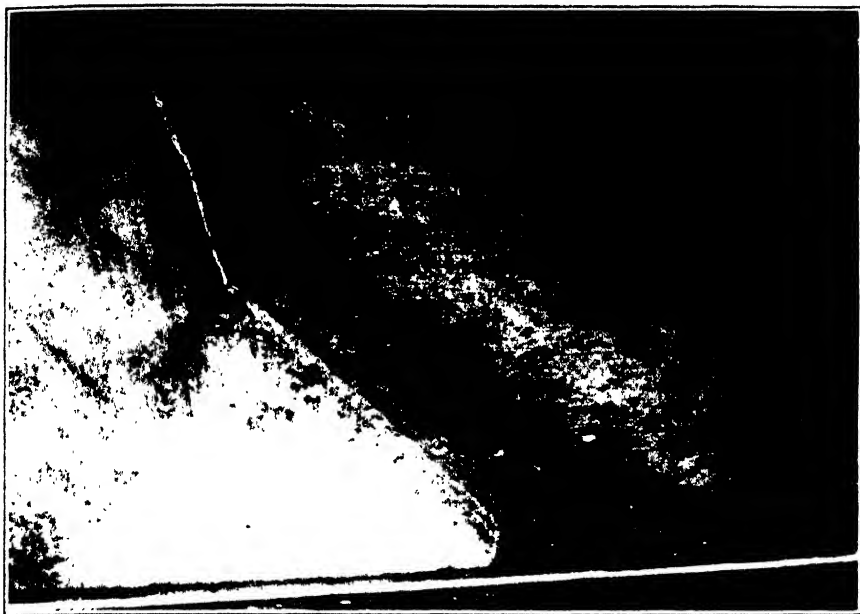


FIG. 1. From an aerial photograph showing irregular streaks of cotton plants killed by root rot lying parallel to a desert wash. For many years before the land was cleared of native vegetation the wash had served as a waste channel for river flood water escaping from irrigated fields some of which were infested with the root-rot fungus. The dark area nearest the channel represents nonsusceptible weeds, mostly *Portulaca oleracea*. The irregular high streaks represent the dead areas of cotton plants that, in the photograph, are continuous for about  $\frac{1}{4}$  mile. They are bordered by healthy areas at the extreme right and left.

Further explorations in the watershed areas of some of the stream beds of the Southwest may disclose the fact that root rot is not uncommon at the higher elevations. Transmission of infected roots and of sclerotia from these higher areas by erosion and drainage affords a possible explanation of some of the infestations in the cultivated areas of lowlands and deltas.—C. J. KING, CLAUDE HOPE, and E. D. EATON, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

*Control of Apple Sooty Blotch by May and June Sprays.*—Sooty blotch of apples is not generally considered a very destructive disease, but in some seasons it produces a serious blemish on apple fruits. The fact that the disease is not evident until late in the season (August) has led to the opinion that late-summer sprays are necessary to its control. However, in spraying tests conducted at La Fayette, Indiana, in 1928 and 1932, it was controlled when the last fungicidal spray was applied in early June.

This period corresponded to the time of maturity and dissemination of the spores of the fungus.

The data<sup>1</sup> reported here were collected incident to spray tests for the control of apple scab and codling moth conducted by the Department of Horticulture of the Purdue University Agriculture Experiment Station. The experimental orchard consisted of 175 19-year-old, normally productive apple trees. A row of persimmon trees and an open woods bordered the east side of the orchard. Branches of the persimmon trees were heavily infected with sooty blotch and possibly were the chief sources of inoculum.<sup>2</sup>

In 1928, the experimental orchard was divided into 2 plots, which contained approximately equal numbers of Jonathan, Stayman, Grimes, and Rome Beauty trees. One plot was sprayed, receiving a pink, calyx, and first-cover spray of liquid lime sulphur and lead arsenate, and a second and third cover spray of lead arsenate and lime. The liquid applications averaged 5½ gallons per tree. The other plot received 12 applications of an 85-15 dust (85 per cent colloidal sulphur and 15 per cent lead arsenate), of which 7 were applied to both sides of the trees. The dust was applied at night at the application rate of 1 lb. per tree. The last fungicidal spray was applied on June 9 and the last dust on August 11, with 4 dust applications in June. One Rome Beauty and 1 Jonathan tree were left without treatment.

At the end of the season apples were counted from 2 Rome Beauty, 2 Stayman, 1 Grimes, and 1 Jonathan tree in the sprayed plot and from 2 Rome Beauty, 1 Stayman, 2 Grimes, and 1 Jonathan in the dusted plot and from the Rome Beauty and Jonathan checks. The fruit from the 4 varieties in the sprayed plot averaged 87.3 per cent clean, while the dusted plot averaged 67.3 per cent. The blotched fruits from the sprayed plot were less severely infected than those from the dusted plot. Only 8.5 per cent of the fruit from the Jonathan check and 2.7 per cent from the Rome Beauty check were clean and many of the infected fruits were nearly blackened by the fungus coating.

In 1932, 2 Stayman trees sprayed with Flotation sulphur, 3 Staymans sprayed with Ansul sulphur, and 1 Rome Beauty sprayed with Bordeaux mixture were selected for sampling from the plots that received applications of the respective sprays at the pink, petal-fall, and 3 and 5 weeks after petal-fall stages. Lead arsenate was used throughout as the insecticide, except for the first and second cover sprays in the Flotation-sulphur plot,

<sup>1</sup> Data for 1928 from: Zaring, Doyle, "Studies on sooty blotch of apple." Thesis for degree of M.Sc. in Agriculture, Purdue University, 1929. Copies on file in Library and in Department of Horticulture of Purdue University.

<sup>2</sup> Recent tests by the writer have shown that the disease can be produced on excised green apples in a moist chamber by atomizing with a spore suspension from a culture of the sooty-blotch fungus isolated from persimmon.

where Dutox and Verdol were substituted. A Rome Beauty and a Stayman tree, sprayed with liquid lime sulphur 1-50 in the pink and petal-fall stages only, served as checks on the 2 later applications of fungicides.

From the trees sprayed with Bordeaux mixture, Flotation sulphur, and Ansul sulphur, 99.5, 97.9, and 94.1 per cent of clean fruit, respectively, were obtained, while 37.5 and 57 per cent of clean fruit were secured from the respective Rome Beauty and Jonathan check trees.

The 1928 and 1932 results indicate that effective control of sooty blotch can be obtained if fungicides are applied just prior to the limited period of spore dissemination. The last fungicidal sprays were applied on June 9, 1928, and June 13, 1932.

Baines and Gardner<sup>3</sup> observed that spore liberation occurred during a rain on June 5 and 6, 1931. On May 31, 1932, many immature and few mature spores were observed on the stems or branches of 8 of the following 10 hosts: leatherwood, smilax, willow, false bitter sweet, prickly ash, sassafras, sumac, bladder nut, maple, and hawthorn. On June 11 an abundance of mature spores were observed on all of the above plants. Microscope observations indicated that heavy spore dissemination from all of these hosts apparently was limited to the rainy period of June 15 to 18, 1932.—RICHARD C. BAINES,<sup>4</sup> Department of Botany, Purdue University Agricultural Experiment Station, La Fayette, Indiana.

*Elimination of Potato Late Blight from North America.*—Species of tuberous solani exist in the highlands of central Mexico that are immune from the late blight or mildew caused by *Phytophthora infestans* (Mont.) de Bary. The following species are known to possess this immunity: *Solanum demissum* Lindley, *S. cogoacanum* Bukasov, *S. neoantipoviczii* Bukasov, *S. antipoviczii* Bukasov, *S. verrucosum* Schlecht. (in part), *S. polyadenium* Greenm., *S. sambucinum* Rydb., *S. bulbocastanum* Dunal, *S. ajuscoense* Bukasov. None of the species is of commercial importance. The plants are not large, the stolons are long (up to 2 m.), the tubers vary in size from 1 to 5 cm., and none of the species begins tuber production until the length of day is 12 hours or less. Furthermore, cross-fertilization between some of these species *inter se* or with varieties of the cultivated potato has not yet been accomplished, although all of the species are self-fertile. Curiously enough, *S. demissum* with 72 chromosomes (diploid)<sup>5</sup> has given the most hybrids with cultivated sorts (48 chromosomes).

<sup>3</sup> Baines, R. C., and M. W. Gardner. Pathogenicity and cultural characters of the apple sooty-blotch fungus. *Phytopath.* 22: 937-952. 1932.

<sup>4</sup> The writer wishes to express his appreciation to C. L. Burkholder of the Department of Horticulture of Purdue University, for his cooperation in securing the data presented here.

Hybrids in which *S. demissum* is the male parent, however, are rare because fertilization is not accomplished. On the other hand, a generous quantity of seed can be obtained with *S. demissum* as the female parent. The difficulty here is to find domesticated sorts that produce good pollen and, particularly, commercial varieties that possess this ability. The new variety Katahdin and some of its sibs and some unnamed varieties developed by F. A. Krantz in Minnesota really have made possible a favorable outcome of an endeavor that, at the outset did not offer much encouragement.

At Ithaca, thus far, *S. demissum* as pollen parent has given a very scant set of seeds only 5 times and each time on variety Rural or on Rural hybrids. The first-generation hybrids are intermediates in many characters, are all blight-immune and are practically male-sterile. Less than 100 individuals of the second generation have been produced. A segregation occurs and blight immunity is found to be heritable, although it is not possible to determine the probable mode from such meager material. Also, some of the progeny have the general appearance of the Rural parent with fairly short stolons and tuber production before autumnal equinox.

When *Solanum demissum* is the female parent and fertile pollen from cultivated varieties is employed, the first-generation hybrids usually resemble the wild parent rather closely, although some intermediate characters may be observed. Practically all of the plants of this generation are immune from blight, (90 per cent at present, but some of the *demissum*-like parents are of questionable genetic purity), they develop long to very long stolons, and a good many of them persist in the habit of producing tubers only with short day. Such hybrids are mostly fertile. In the second and succeeding generations (at least as far as the fourth) all plants tested have reverted to the wild type; all are immune from blight and continue so in succeeding generations.

If an immune first-generation plant is backcrossed with pollen from a cultivated variety the progeny is heterogeneous. Subject to the reservations noted above, 68 per cent (1762 plants tested) of the backcross plants are immune from blight, most of them are much larger than *demissum* and some are larger than either parent, the stolons tend to be decidedly shorter than in the first generation, and a fair proportion of the plants produce tubers approximating commercial size and before the equinox. As the plants grow in the field their *demissum* parentage is noticeable especially in foliage and flower.

It is not known yet what is to be expected from a selfed backcross, but when a blight-immune backcross plant is again backcrossed with pollen from a cultivated variety the progeny is again heterogeneous, this time inclining in general aspect to the cultivated type. Records exist for 767 such double backcross plants and of these 50 per cent are immune from

blight. About 200 blight-immune double-backcross plants grown from seeds started on May 15, 1933, were set in the field on June 30 and subjected to repeated natural infection in late September and early October. No infection occurred, nor was it expected, because of the severe elimination tests in the greenhouse in mid-June. From this lot a dozen plants were selected that had the appearance of domestic varieties and which bore from 6 to 9 tubers of commercial size, smooth and white, with fleet eyes, and on short stolons. About a dozen other selections were made of plants approximating commercial types and size and from previous trials with seedling plants it may be confidently predicted that several of them will yield tubers of larger size in 1934.

Many of the double-backcross plants set seed freely when selfed, so that it will be known soon what such selfings will yield; and also whether these plants can be used to transmit blight immunity to the well established commercial varieties practically all of which are male-sterile.

While it is felt that the title of this note is justified as a not too distant possibility, it is recognized fully that the development of these new blight-immune varieties may serve merely to demonstrate the existence of clear-cut biologic specialization of *Phytophthora infestans* on the potato in North America, just as similar work in Germany has brought out the existence of biotypes in that country. A summary of the situation in North America has been published recently in another place.<sup>1</sup> The collaboration of interested persons is sought not only to determine the integrity of the blight immunity of these varieties throughout the late-blight area but also to determine the adaptability of the varieties for commercial production. Small samples for experimental purposes should be available in 1935, assuming that a disease that has become prevalent recently at Ithaca does not ruin everything. This disease is apparently the one described by Barrus<sup>2</sup> under the name "streak." It had not been observed again at Ithaca, except perhaps sporadically, until the summer of 1932. Every effort was made to eliminate the disease by tuber indexing and roguing in 1933 but without success. Such incidents merely emphasize the importance of disseminating widely any likely new varieties at the earliest possible time.—DONALD REDDICK, Cornell University, Ithaca, N. Y. (Cooperative work with the Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture).

<sup>1</sup> Reddick, Donald, and Willard Crosier. Biological specialization in *Phytophthora infestans*. Amer. Potato Jour. 10: 129-134. 1933.

<sup>2</sup> Barrus, M. F. Physiological diseases of potatoes. Ann. Rept. Quebec Soc. Prot. Plants, 9: 45-53. 1917. Streak is described on p. 51 and the 3 illustrations refer to this disease.

*Verticillium Wilt of Cotton in Greece.*—The wilt caused by a species of *Verticillium*, commonly designated as *Verticillium albo-atrum* R. & B., was first recorded as occurring on cotton under field conditions by Sherbakoff<sup>1</sup> in Tennessee, in 1928. The following year he recorded its discovery on the opposite side of the Mississippi River in Arkansas.<sup>2</sup> The disease was noted in 1931 by Rudolph<sup>3</sup> on cotton from California and from Mississippi by Miles and Persons<sup>4</sup> in 1932.

Recent correspondence between the author and Dr. J. A. Sarejanni, Plant Pathologist at the Institut Phytopathologique Benaki, Kiphissia-Athenes, Greece, has indicated that the trouble has recently made its appearance in that country. The following is a translated excerpt from a letter from him dated February 22, 1933:

“In the month of September of last year I was called to the region of Copias to diagnose a new disease of cotton. I was able to establish that it was a question of wilt or hadromycosis. I was, however, greatly astonished to see appear from twenty cultures nineteen of pure *Verticillium* and a single one of *Fusarium*. Happily, however, your recent article with Mr. Persons,<sup>4</sup> which arrived soon after, cleared me up on the matter.

“These two hadromycoses or wilts, that of the *Verticillium* as well as the *Fusarium*, were observed at this time for the first time in Greece. I question if they have not come from America, since I have never found them except in fields planted with seed imported from Carolina in the United States in 1932. Is it possible that *Verticillium* wilt of cotton is perhaps more widespread in the United States than has been believed?”

In replying to this letter a request was made that cultures of the *Verticillium* isolated from cotton in Greece be sent for comparison with those isolated from cotton in Mississippi. These cultures have recently been received and are identical in growth characters of the colonies and in morphological details of the fungus with those secured from cotton in Mississippi and also with those cultured from cotton in Tennessee by Sherbakoff in 1928.

The following excerpt from a letter from Sarejanni, dated January 24, 1934, shows the present status of the disease in Greece:

“The hadromycosis (wilt) due to *Verticillium* has spread throughout the whole of Greece in the course of this year. I have isolated it in the north in Macedonia as well as in the south in continental Greece. From all the isolations which I have made this year from various specimens drawn

<sup>1</sup> Sherbakoff, C. D. Wilt caused by *Verticillium albo-atrum*. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Sup. 61: 283–284. 1928. (Mimeographed.)

<sup>2</sup> Sherbakoff, C. D. *Verticillium* wilt of cotton. Tenn. Agr. Exp. Sta. Circ. 24: 2. 1929.

<sup>3</sup> Rudolph, B. A. *Verticillium* hadromycosis. *Hilgardia* 5: 197–354. 1931.

<sup>4</sup> Miles, L. E., and T. D. Persons. *Verticillium* wilt of cotton in Mississippi. *Phytopath.* 22: 767–773. 1932.



from different localities I have always secured *Verticillium* and never *Fusarium vasinfectum*, which latter I have isolated but a single time last year from Livadia.

"From the simultaneous appearance of the trouble in several provinces quite distant from each other, as well as from the fact that the appearance has followed the introduction of foreign varieties, I am much inclined to persist in the hypothesis of seed transport. In spite of experiments made in this direction, however, I have not been able to demonstrate the fungus on the interior of seeds borne on diseased plants. . . . I do not believe definitely that the disease has not existed in Greece previous to that date (date of introduction of foreign seed) simply for the reason that it had not been pointed out to me, but I should feel secure in saying that the introduction of American seed has brought about heavy infections in the cotton sections.

"The Cotton Institute recently sent me the following information regarding cotton seed which it introduced from America in 1932.

"Pedigreed Cleveland, 5 tons from North Carolina.

Acala, 20 tons	} These four varieties were furnished by T. W. Wood & Sons, Richmond, Virginia. The point of origin of these seeds was not specified."
Ingold, 10 tons	
Trice, 15 tons	
King, 45 tons	

Experiments of the author and of other workers in this country are in agreement with those of Sarejanni in an inability to demonstrate the occurrence of the organism within the cotton seed. These experiments, however, have not been extensive enough to warrant a conclusion that the disease may not, at least sometimes, be transmitted in that manner. There exists the hypothesis, suggested by Sarejanni in other correspondence, that the organism occurs, not within the seed, but on the seed held by the fuzz or lint with which it is covered. There is also the possibility that the organism was already present in the soils of Greece, possibly attacking, or present without evident damage on, some other host plants, and that the cotton commonly grown in Greece is immune from or resistant to its attack. The varieties of cotton imported from the United States may have been readily susceptible to the attacks of the Grecian strain already present, thereby presenting what would seem to be evidence in support of the assumption that the causative organism had been introduced on such imported seed. L. E. MILES, Mississippi Agricultural Experiment Station, State College, Mississippi.

*Variation in Hyphal-tip Cultures from Conidia of Helminthosporium gramineum.*<sup>1</sup>—Hyphal-tip cultures isolated from individual germinating conidia were used in a study of variation in *Helminthosporium gramineum* Rabh. Each isolant, containing at least one cell with a septum, was severed from the advancing germ tube. Colonies grown from hyphal tips, isolated

<sup>1</sup> Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

directly from conidia produced on striped barley leaves, minimized the artificial culture effects on the fungus. This also made possible the observation of some of the variations that most probably existed in the conidium at the time of germination. A series of isolations involving from 2 to 5 hyphal tips from each of 5 conidia was made in October, 1932, and a second series, involving hyphal tips of 20 additional spores, was made in August, 1933. The hyphal-tip cultures have been studied on media and in inoculation trials.

Hyphal-tip cultures from the same conidium reacted differently in Petri dish culture on potato-dextrose agar, in pathogenicity tests, and in symptoms produced on the barley plants. The type of mycelial growth and pigmentation varied widely in hyphal-tip cultures from a conidium. These cultures from the same spore were found to vary from almost nonpathogenic to highly pathogenic when seed inoculations were made on Wisconsin Pedigree 5-1 barley, grown in 1932. The cultures from some spores showed less pathogenicity range than did others. The disease symptoms produced by cultures from certain conidia showed further variations. The striped plants produced by inoculations with some hyphal-tip cultures developed characteristic leaf lesions on plants that were essentially the same height as the controls. The diseased plants from inoculations with other cultures from the same spore were mostly dwarfed and rosetted. The infected plants from cultures of other spores developed both types of striped plants. Thus it is apparent that a conidium may carry within its cytoplasmic and nuclear composition more than one cultural type, and more than one pathotype. There is evidence that some cultures are more stable than others, as repetition of pathogenicity trials has shown some cultures to be more consistent in producing the same percentage of stripe and type of symptoms. However, a hyphal-tip culture of *Helminthosporium gramineum* seemingly does not necessarily guarantee purity and stability of characters. Limited monohyphal reisolations in one of the more stable cultures have indicated that the new isolants resembled the parent rather closely, which suggests that a condition of stability in cultural characters, pathogenicity, and disease symptoms is being approached.

Cytological preparations in which Flemming's fixative and Heidenhain's Haematoxylin stain were used have shown germinating conidia, germ tubes, and mycelial cells to be multinucleate. With the multinucleate condition prevalent in the conidium, heterocaryosis may possibly exist. This might serve as a basis for explaining variations found in cultures obtained by the isolation of germ-tube hyphae from the same conidium. The results of pathogenicity, cultural, physiological, and cytological investigations of hyphal-tip cultures will be reported more completely at a later date.—H. L. SHANDS and JAMES G. DICKSON, Plant Pathology Department, University of Wisconsin, Madison, Wisconsin.

## BOOK REVIEW

*The Efficacy and Economic Effects of Plant Quarantines in California.*  
By H. S. Smith, E. O. Essig, H. S. Fawcett, G. M. Peterson, H. J. Quayle,  
R. E. Smith, and H. R. Tolley. University of California Bulletin 553:  
276 pp. 1933.

The American reading public—even the scientific public—still shows great respect for board covers and cloth bindings, and particularly for those volumes that are sold by great commercial publishing houses. It is possible then that the book under review, which comes to us as Bulletin 553 of the California Experiment Station, may not receive the general attention it richly deserves.

Two economists, 2 plant pathologists, and 3 entomologists, each of recognized standing in his own field, collaborating over a period of 3 years, have produced a volume that contains a discussion of the social-economic aspects of changes in crop volume, whether caused by disease or otherwise. This discussion is clearly stated in nontechnical language and can be read easily and profitably by anyone really interested in these questions. It nevertheless deserves the thoughtful consideration of those professionally interested in the control of plant pests and diseases.

Since the seven authors form a committee appointed for the specific purpose of studying the "Efficacy of plant quarantines as applied to California and their economic effects" there, it is inevitable that much of the matter will be of interest only to limited groups. The section on "Administrative aspects of plant quarantine," pages 82–99, will be read chiefly by those professionally interested in quarantine problems. This is true in even greater degree of the section that deals with the history and administration of plant quarantines in California, pages 104–139. Most of the analyses of the specific quarantines now in force in California, 38 in number, will probably be read carefully only by students of the particular insects or diseases involved.

It is in the introductory sections, particularly in the sections on economic aspects, that the most stimulating material is found. In his foreword, Dean Hutchison, who appointed the committee, advances the belief that, in view of the national and international relations, it would have been desirable to have had the study undertaken by some national agency, preferably one with no connection with either Federal or State governments. He says also that he sought and failed to get such assistance. The committee, however, has included much matter of general significance. Following the foreword, there is a discussion of the biological aspects of plant quarantine in which is included a general review of the distribution of

plant pests and diseases and the methods of their dispersal, the probability of introduction, of establishment, and of their becoming important. This matter, while well stated and appropriately included, is in large part well known and generally admitted.

When, on page 42, the committee attacks the question of the effect of pests on the total social income and on the distribution of income among individuals and groups they enter ground too often avoided or slighted, but of fundamental importance not only to biologists but to the general public.

Specific illustrations are given of the *immediate* effects on growers of changes in volume of crop (p. 51) and its complexity summarized in the statement:

"Sometimes the growers as a group or class might gain, in other cases they would lose—a small change in volume may have one tendency, a large change just the opposite."

Particularly refreshing in view of the recent tendency in some quarters to regard disease control as of concern only to the producers, are the conclusions of the committee when it comes to consider the broader aspects of losses from diseases or pests, which may be best stated in quotation:

"Society considered as a large group of producers and consumers has interests almost identical with those of individual producers."

"In the long run the consumers tend to pay all of the costs and to receive all of the benefits [of pest and disease control measures] except when some form of monopolistic control intervenes and then consumers continue to pay all of the costs but do not receive all of the benefits."

*"The consumer always benefits from an abundance of production and should be more interested in maintaining plant quarantines that insure such abundance than growers as a group."*

The italics are in the original, though I should be glad to accept the responsibility for their use. And, if it were permissible in a review, I should move to amend by striking out "Maintaining plant quarantines" and inserting "All plant-pest and disease-control measures."

On the validity of the committee's conclusions as stated in the last ten pages, I do not feel competent to pass. As would be expected of skilled and experienced investigators, the authors fully realize that they are not saying the last word. Indeed, to quote again from Dean Hutchison's foreword—"The committee feels that it has made only a beginning." May I be permitted to add that it is a good beginning.—NEIL E. STEVENS, Bureau of Plant Industry, Washington, D. C.

# REPORT OF THE TWENTY-FIFTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

## THE BOSTON MEETING

The twenty-fifth annual meeting of The American Phytopathological Society was held from December 28 to 30, 1933. Approximately 150 members were present and 67 papers were presented at 7 sessions. There were joint sessions with Section G, with the Mycological Society of America, and the Potato Association of America.

The contributions presented at this meeting may be classified according to subject as follows: Taxonomy, 11 papers; virus diseases, 9 papers; diseases of vegetables, tobacco, cotton, and other crops, 13 papers; diseases of fruits, 9 papers; diseases of cereals and forage crops, 8 papers; diseases of ornamental and forest trees, 9 papers; and potato diseases, 8 papers.

The annual conference on extension work in plant pathology, held December 28 under the chairmanship of Charles Chupp, was devoted to a discussion of the following subjects: (1) New seed disinfectants and fungicides, (2) commercial seed treatment, its advantages and limitations, (3) seed treatment and other service work in the counties, (4) emergency-garden relief work, (5) new points on illustrative material and its use.

A session on pathological activities in emergency and recovery programs, under the chairmanship of A. F. Woods, brought to light the important part that plant pathologists are at present taking in the national recovery programs and called attention to opportunities for plant pathologists to render service under present economic conditions.

On December 28 was held the annual dinner, entertainment being supplied by Wm. H. Weston, Jr. (Harvard University), W. L. Doran (Massachusetts State College of Agriculture), F. A. Wolf (Duke University), and Fred Sparrow (Dartmouth College).

Abstracts of the papers on the program of this meeting are printed in the January number of PHYTOPATHOLOGY.

## OFFICERS AND REPRESENTATIVES

The following officers and representatives were chosen:

*President*, N. E. Stevens, U. S. Department of Agriculture, Washington, D. C.

*Vice-President*, G. W. Keitt, University of Wisconsin, Madison, Wis.

*Councilor* (two years), F. D. Heald, State College of Washington, Pullman, Wash.

*Associate Editors* (three years), Glenn G. Hahn, Yale University, New Haven, Conn.; R. P. White, State College of Agriculture and Mechanic Arts, New Brunswick, N. J.; F. O. Holmes, The Rockefeller Institute for Medical Research, Princeton, N. J.; and J. J. Christensen, University Farm, St. Paul, Minn.

*Business Manager* (one year), F. C. Meier, U. S. Department of Agriculture, Washington, D. C.

*Advertising Manager* (one year), R. S. Kirby, Pennsylvania State College, State College, Pa.

*Representatives on the Council of the A. A. A. S.* (one year), N. E. Stevens, U. S. Department of Agriculture, Washington, D. C., and H. S. Cunningham, Long Island Vegetable Research Farm, Riverhead, N. Y.

*Member of the Board of Governors Crop Protection Institute* (3 years), J. F. Adams, University of Delaware, Newark, Del.

*Elector to Division of Biology and Agriculture of the National Research Council*, John W. Roberts, U. S. Dept. Agr., Washington, D. C.

The following temporary committees were appointed to serve throughout the meetings:

*Auditing Committee*, John W. Roberts and John Stevenson.

*Committee on Elections*, A. N. Brooks and H. S. Cunningham.

*Committee on Resolutions*, W. H. Tisdale, Annie R. Gravatt, and S. M. Zeller.

#### REPORT OF THE SECRETARY-TREASURER, 1933

The Society started the year 1933 with 827 members. During the year 5 former members were reinstated, but we lost 84, of which number 21 resigned, 3 died, and 60 were dropped from the Society for nonpayment of dues. Each of the 60 suspended received 3 statements and a letter but there was no response to any of our communications. Thus, at the beginning of the winter meetings, our membership stood at 748. Election of the 26 new applicants for membership brought our membership to 774 for the beginning of 1934. Of this number 100 are paid up life members and 70 are life-sustaining.

#### STATEMENT OF ACCOUNTS FOR THE YEAR ENDING NOVEMBER 30, 1933

##### *Receipts:*

Balance from 1932		\$1,849.18
Annual Dues:		
1930	\$ 5.00	
1931	5.00	
1932	34.40 (\$ 10.00 life)	
1933	1,661.02 ( 259.85 life)	
1934	1,358.69 ( 380.00 life)	
1935	22.05 ( 10.00 life)	3,086.16
Interest on checking account		9.53
Donations to Lyman Fund included in checks for dues		7.86
Sales and special sales in checks for dues		5.95
Classics included in check for dues		0.75
Checks to replace those returned by bank due to bank holiday, etc.		65.00
Refund by Sec.-Treas., expenses of Atlantic City meeting		3.88
Cash from B. B. Mundkur for dues in other societies		7.15
Share of A. P. S. in distribution of surplus funds of International Congress of Plant Sciences		179.58
Total Receipts		\$5,215.04

##### *Expenditures:*

Member subscriptions transferred to PHYTOPATHOLOGY (1933, part 1932)	\$2,532.52
Transferred to Sinking Fund savings account	395.00
Secretarial work	177.56
Printing (preliminary programs, ballots, etc.)	66.53
Postage and stamped envelopes	75.70
Telephone and telegraph	17.33
Advance of funds to Secretary-Treasurer for Atlantic City meeting	50.00
Expenses of Centennial Exhibits Committee	11.00
Checks returned by bank and discount on check	81.70

Donations transferred to Lyman Fund . . . . .	7 86
Sales and special sales transferred to PHYTOPATHOLOGY . . . . .	5 95
Classics transferred to H. H. Whetzel . . . . .	0.75
Dues in other societies, B. B. Mundkur . . . . .	7.00
Supplies . . . . .	1.00
Federal tax on checks . . . . .	0.64
Share of cost of electrical transcription . . . . .	2.50
Balance on hand . . . . .	1,782.00
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Total Expenditures . . . . .	\$5,215.04

## SINKING FUND

The Sinking Fund, obtained by deducting \$5.00 (formerly \$6.00) from each life-sustaining membership payment, now stands at \$7,986.00, of which \$5,000 is invested in first mortgage notes. \$2,781.00 is deposited in a savings account with the Riggs National Bank of Washington, D. C. The remainder, \$205.00, has not yet been transferred from the checking account of the Society. Income from this fund now amounts to almost \$375.00 and is used to help defray the cost of publishing PHYTOPATHOLOGY.

Respectfully submitted,

F. C. MEIER, *Secretary-Treasurer.*

## REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1933

## STATEMENT OF ACCOUNTS FOR THE YEAR ENDING NOVEMBER 30, 1933

*Receipts:*

Balance from 1932 . . . . .	\$1,183.79
Subscriptions:	
1931 . . . . .	\$ 1.24
1932 . . . . .	24.83
1933 . . . . .	2,311.36
1934 . . . . .	305.61
Member subscriptions, 1932 and earlier . . . . .	32.52
Member subscriptions, 1933 . . . . .	2,500.00
Sales of back volumes and numbers . . . . .	106.05
Special half-price sale . . . . .	208.15
Advertising:	
1932 . . . . .	\$ 87.16
1933 . . . . .	417.20
Interest on Sinking Fund . . . . .	342.45
Checks to replace those returned by bank during bank holiday, etc. . . . .	50.37
Dues in The American Phytopathological Society included in checks with subscriptions . . . . .	15.00
Dominico Pirone for Phytopathological Classic No. 2 . . . . .	200.00
Association of Hawaiian Pineapple Cannery for share of cost of Carter articles . . . . .	125.00
Rockefeller Institute for cost of Price article . . . . .	106.27
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Total Receipts . . . . .	\$8,017.00

*Expenditures:*

## Printing and distributing PHYTOPATHOLOGY:

Vol. XXII, No. 12 .....	\$378.31	
Index to Vol. XXII .....	182.50	\$ 560.81
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Vol. XXIII, No. 1 .....	\$621.62	
No. 2 .....	540.98	
No. 3 .....	630.20	
No. 4 .....	541.53	
No. 5 .....	549.95	
No. 6 .....	366.14	
No. 7 .....	343.80	
No. 8 .....	338.78	
No. 9 .....	374.15	
No. 10 .....	573.04	4,880.19
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Postage .....	447.29	\$5,888.29
Secretarial work for Business Manager .....		176.06
Expenses of Office of Editor in Chief .....		292.73
Commission of former Advertising Manager .....		40.91
Part commission of present Advertising Manager .....		42.00
Expenses of Advertising Manager .....		26.80
Stamps and envelopes .....		102.54
Printing .....		46.98
Transferred to The American Phytopathological Society for dues .....		15.00
Checks returned by bank and exchange charge .....		68.30
Telephone and telegraph .....		4.26
Subscription refunded .....		5.53
Printing Classic No. 2 .....		186.84
Incidental expenses, handling Classic No. 2 .....		17.27
Binding PHYTOPATHOLOGY for official set .....		34.65
Federal tax on checks .....		0.80
Supplies .....		0.40
Balance on hand .....		1,067.64
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Total Expenditures .....		\$8,017.00

At the end of the year 1932 there were 536 subscribers (nonmembers) to PHYTOPATHOLOGY, including 7 complimentary subscriptions. Fifty-one (of which 27 were from U. S. S. R.) were cancelled for 1933 and during the year 32 were dropped for non-payment of subscriptions. With the addition of 49 new subscribers, the net loss was 34, reducing the list to 502.

*Note:* In order to facilitate preparation of the annual report at a time when his work in connection with the Society is always heavy, the Secretary-Treasurer of The American Phytopathological Society, who is also Business Manager of PHYTOPATHOLOGY, closed the accounts for both Society and Journal at the end of November this year. This makes the apparent receipts and expenditures considerably less than would normally be the case for a full year, as more business is transacted during the first 2 weeks of December than in any other similar period of the year.

Respectfully submitted,

F. C. MEIER, *Business Manager.*



## REPORT OF THE COMMITTEE ON LYMAN MEMORIAL FUND

The Lyman Memorial Fund has grown during the period, January 1, 1933, to December 31, 1933, from \$1,679.77 to \$1,870.47. This includes contributions of \$138.86 and interest amounting to \$51.84. The Fund has, therefore, grown by about \$200.00 during the past year.

One of the principal features was the fact that the members of the Plant Pathology Department, of the New Jersey Experiment Station, contributed \$63.00. The Committee wishes to make public acknowledgment and thank the members of the Department for their generosity.

No particular drive was made for contributions, because of the economic conditions. Neither were any munificent bequests obtained. Nevertheless, the Fund has grown gradually and steadily, even though rather slowly, and the Committee feels that efforts should be continued and intensified as soon as improvement in economic conditions warrants.

Respectfully submitted,

E. C. STAKMAN, *Chairman.*

## REPORT OF THE EDITOR IN CHIEF

In my report submitted a year ago, at Atlantic City, reference was made to the effect of the "depression" on the printing budgets of plant-science journals and the general absence of any diminution in the tide of manuscripts submitted for publication in PHYTOPATHOLOGY. Again, even though, as some assert, we have not yet sighted the cloud of silver lining, the volume of manuscripts submitted to your editorial board has shown no sign of depression. Indeed, during the 6-year period, 1928 to 1933, the rate of arrival of manuscripts on the editor's desk has increased from one manuscript every 4 days to one every  $2\frac{1}{2}$  days.

Somewhat contrary to expectation, this increase in volume has been marked by a general improvement in the scientific and literary merit of the several manuscripts. During the past year, particularly, the improved excellence of papers contributed has been notable and encouraging. The members of the editorial board have, however, found it necessary to recommend drastic revision of a number of manuscripts, and compliance with their recommendations has, with very few exceptions, been cheerfully accorded. Whatever may have been their just deserts, your editors have received among several epistolary bouquets very few of the anticipated prickly-pear variety. It would prove salutary to the editorial staff if your modesty would give way occasionally to some constructive criticism, for PHYTOPATHOLOGY is *our* journal and the editors desire above everything else to make it fully worthy of the scholarship and the best scientific effort of our membership.

The recommendation proposed and adopted by the Society in business session at the Atlantic City meeting, calling for the preparation of author abstracts of manuscripts submitted for publication in PHYTOPATHOLOGY, has been in practical operation for the past several months. Abstracts thus far submitted have been forwarded to BIOLOGICAL ABSTRACTS for publication in that journal.

The 23rd volume of PHYTOPATHOLOGY contains 1006 pages of printed text and illustrations, or 4 pages more than comprised Volume 22. The 1006 pages of our journal for 1933 are classified as follows: 78 articles, 36 phytopathological notes, 2 reports, 7 book reviews, 124 abstracts (2 by title only), and 212 text figures. During the period January 1 to December 31, 1933, inclusive, approximately 153 manuscripts of articles, phytopathological notes, reports, book reviews, etc., and 64 abstracts (1 by title only) were submitted. Of this number 12 were returned to their authors for revision, 12 were found unacceptable, and 5 were withdrawn by their authors. Of the manuscripts re-

ceived in 1932, too late for publication that year, 2 were returned, in the current year, for revision. In addition to the several papers published this year, 9 articles, 11 phytopathological notes, 1 book review, and 57 abstracts (1 by title only) are now (December 31) in press. The index for Volume 22 was published as a supplement to the February, 1933, number of PHYTOPATHOLOGY.

The editor again wishes to express his appreciation and gratitude in behalf of the excellent clerical and editorial services rendered by Frances W. Todd. The thanks of the editor are here extended to the several members of the editorial board who have given so much of their time and effort to duties incident to the publication of our journal and to The Science Press Printing Company for its high-grade workmanship in printing PHYTOPATHOLOGY.

Respectfully submitted,

H. B. HUMPHREY, *Editor in Chief.*

#### REPORT OF THE BUSINESS MANAGER, PHYTOPATHOLOGICAL CLASSICS

I beg to submit herewith the annual financial report covering the receipts and expenditures in connection with my stewardship as manager of Phytopathological Classics for the year 1932-33.

Total number of Classics No. 1 <i>received</i>	647
Total number of Classics No. 1 <i>sold</i>	196
Balance on hand	451
Total number of Classics No. 2 <i>received</i>	1012
Total number of Classics No. 2 <i>sold</i>	330
Total number of Complimentary copies	26
Balance on hand	656
Total number of Classics No. 3 <i>sold</i>	2

The above classics were sold in the following manner:

Classic No. 1	— 19 copies at \$ .50	\$ 9.50
Classic No. 2	— 153 “ “ .50	76.50
Classic No. 1 and 2	— 174 “ “ .75	130.50
Classic No. 1 and 2	— 1 copy “ .74	.74
Classic No. 1, 2, & 3	— 2 copies “ 1.25	2.50

Total receipts from sale of classics	\$219.74
Balance from Pirone check	\$ 13.16
Total receipts	\$232.90

Expenditures itemized:

Express charges	\$ 1.69
Envelopes and letterheads	16.00
Advertising, reprints	5.58
Postage	21.38

Total expenditures	44.65
Balance of money on hand	\$188.25

In December of last year I received from the Secretary of The American Phytopathological Society the residue copies of Classic No. 1, a total of 647. I also received from his hands the total number of Classic No. 2, 1012 copies. Classic No. 3, "The Discovery of Bordeaux Mixture," 3 papers by Millardet, translated by F. J. Schneiderhan, are now in press and will be ready for distribution at the Boston Meeting at 50 cents each. The above report is of December 8. You will note that on this date 2 advanced sales of Phytopathological Classic No. 3 were made, but as Classic No. 3 was not then off the press the cost of same does not appear in the items of expenditure, for the past year, but will appear next year.

Although the total of Classics No. 1 and No. 2 sold during the past year is not extraordinarily large, being 196 for Classic No. 1 and 330 for Classic No. 2, still, the net income from these sales including 2 copies of Classic No. 3, totaled \$188.25. This sum is much more than sufficient to pay for the publication of 1000 copies of Classic No. 3. I should recall to you that the cost of publication of Classic No. 2 was donated by Mr. Dominico Pirone, father of Dr. P. P. Pirone, the translator. In fact, there was an item of \$13.16 cash balance from the check sent me by Mr. Pirone after the cost of publication of Classic No. 2 had been met. This \$13.16 is also included in the net \$188.25.

You will be interested to know that manuscript for Classic No. 4 is nearly ready for publication. It is a translation of Woronin's paper on Club Root of Cabbage. This has been translated by Dr. Charles Chupp, of Cornell University. The publication of the translation will include, of course, the plates illustrating Woronin's original paper. It is expected that the income from the sales of Classic No. 3, with additional sales of Classics No. 1 and No. 2, will provide a sum sufficient to pay for publication of Classic No. 4 sometime early in the coming year. All members of the Society are urged to constitute themselves volunteer agents for the sale of these Classics. A considerable sum of money may be conserved if each member of the Society will undertake to solicit subscriptions to these Classics amongst his friends and colleagues, collect the money, send the order to me, and assume the responsibility of distributing the copies upon their receipt. All such orders for these Classics should be accompanied by money order, and a list of the subscribers with their addresses, in order that we may canvass them later for future issues of these Classics.

The editor and manager solicit suggestions for future Classics in this series. Let us know what you would like to have translated. We shall give your suggestions careful consideration.

Respectfully submitted,

H. H. WHETZEL, *Business Manager,*  
*Phytopathological Classics.*

#### REPORT OF THE ADVERTISING MANAGER

During 1933, a total of 125 advertisements were run in PHYTOPATHOLOGY. These 125 advertisements consisted of a total of 85½ pages. Of the total advertising, 62 advertisements were nonrevenue, including exchange ads with other journals and such Society helps as Phytopathological Classics, back-number sale, meeting announcements, etc.

A study of the 1932 and 1933 advertising records shows the following facts: The income from ads dropped from \$713.05 in 1932 to \$561.50 in 1933, or a drop of 21.2 per cent, and the number of pages of revenue ads dropped from 44½ to 36½, or a 17.3 per cent drop. The number of revenue advertisers increased from 15 in 1932 to 16 in 1933, and the number of revenue advertisements increased from 61 to 63 during the same period. The number of one-page ads during these 2 years decreased from 36 in

1932 to 21 in 1933, while the number of one-half- and one-quarter-page ads increased from 25 in 1932 to 42 in 1933. From these comparisons, it appears that advertising in PHYTOPATHOLOGY is growing.

Respectfully submitted,

R. S. KIRBY, *Advertising Manager.*

#### COMMITTEE ON EDITING PHYTOPATHOLOGICAL ABSTRACTS

The number of abstracts submitted this year was the smallest in the last decade. The Committee deemed it unwise to impose restrictions that would curtail the program still further; accordingly, some liberties with the traditional limitations on the number of contributions acceptable from one group of authors were permitted. It appears that most of the Society membership fully took to heart the restriction against submission of abstracts that were not even intended to be presented at the meetings, but such self-denial was not universal. This tendency to use our abstract series as an outlet for almost immediate publication, and the tendency also to repeat material in the abstracts that has already been, or is soon to be, submitted (in more extended form) for publication—often indeed in PHYTOPATHOLOGY—are the most pernicious features of the present policy of the Society on abstracts. It is manifestly impossible for the Abstract Committee to know who really intends to go to the meetings and present his paper in person, or at least to have a colleague who understands the work that has been done, to substitute for him; and generally the Committee has no means of determining whether publication of the same material *in extenso* has been arranged or is contemplated. Abstinence from this sort of advantage taken of members of the Society who observe the rules, can not be enforced; it can only be requested in the spirit of fair play.

The fact that the regular monthly issues of our journal make provision for the prompt publication of brief articles and preliminary papers (when meritorious) should eliminate all grounds for using the abstracts for any other purpose than giving advance notice and leaving a permanent record of material actually presented on the programs.

The Committee has nothing further to say regarding the quality of the abstracts either as to substance or style. We recognize that they are not of uniform, and some are of very slight, merit. We think there is room for improvement, but there is likewise in the papers that PHYTOPATHOLOGY publishes in full. We regard the abstracts we accept now as not particularly inferior to those published by any other Society, even some very prominent ones. It is amusing at times to listen to the denunciation of the abstract policy of the Society, as well as of the character of the abstracts, so often proclaimed at our annual meetings, in view of the fact that probably none of us, in the eyes of someone else, invariably succeeds in compliance with the scriptural injunction: "Let him who is without sin, cast the first stone."

Respectfully submitted,

FREEMAN WEISS, *Chairman.*

#### REPORT OF COMMITTEE ON FOREIGN PESTS AND PLANT DISEASES

Your committee calls attention to the suggestions made last year that visitors to foreign countries be asked to make reports on foreign pests and diseases, and is pleased to report that W. C. Snyder of California has volunteered to assist in special problems such as potato wart, Colchicum smut, mosaic on Cymbidium, and the Septoria on azalea during sojourn in Europe. We hope that other members who are contemplating foreign travel may undertake similar service.

While the technical publications of foreign countries are readily available and contain much valuable information, there are many features relating to foreign pests

that are especially valuable to our regulatory service but that are not fully covered in these publications. Information of this sort might be obtained most readily by means of such reports as are commonly made on commercial subjects by the Consular Service. It is recommended that the president of the Society make inquiry through the Department of Agriculture as to whether arrangements for obtaining such information can be made.

As a matter of keeping the members of the Society informed on outstanding developments in the quarantine field, it is recommended that the U. S. Department of Agriculture be requested to present annually to the Society a report on the important foreign-pest problems that might be of interest to this country.

After a thorough discussion in this committee with members of the Committee on Quarantine and Regulatory Work regarding the desirability of merging the duties of both committees, it is recommended that this step be taken.

C. R. ORTON, *Chairman*,  
W. A. McCUBBIN,  
F. D. FROMME.

#### REPORT OF COMMITTEE ON QUARANTINE AND REGULATORY WORK

Your committee notes as a feature of considerable significance an increasing interest throughout the world in quarantine matters as reflected in the growing number of regulatory measures recently put into effect in various countries, together with a noticeable tendency to apply more stringent types of restrictions on foreign imports. Several European nations have thus made outstanding advances in their plant-protection systems. Among the most notable recent events in this connection is the establishment of a quarantine and regulatory service in the Republic of China. It may be of interest to members of the Society to note that among other sources of information the U. S. Bureau of Plant Quarantine now contributes a series of circulars summarizing the foreign quarantine regulations imposed by most of the countries of the world.

Among the outstanding recent outbreaks of pests in various countries, which appear to have stirred up considerable apprehension and have exerted a general stimulatory effect toward a tightening up of import restrictions, the following few cases may be mentioned:

A recent outbreak of San José scale in the Balkans is thought to have come from America *via* a European country that has been rather lax in its protective measures.

A spread of potato wart into Switzerland and Portugal is noted and there is now a record of its occurrence in Bolivia. In the case of Switzerland the infection was traced to supposedly immune seed potatoes brought from East Prussia.

There has been considerable spread of the Colorado potato beetle in France, and the alarm of other European countries concerning this pest has resulted in drastic quarantine restrictions against French potatoes. A few of these beetles were recently found in the vicinity of an English port.

A rapid and practically uninterrupted spread of the Dutch elm disease has taken place over Europe and into this country. The strong suspicion that this destructive disease originated in some eastern land and came to Europe in some such fashion as the chestnut blight was brought to America is significant from the quarantine point of view.

Some concern in Europe concerning the disease of firs due to *Rhabdochile pseudotsugae* is finding expression in measures to keep the malady from spreading to continental areas.

Examples frequently quoted to illustrate the damage that may arise from introducing a foreign species into a new habitat are the rabbit and cactus in Australia. The behavior of the muskrat recently let loose in England promises to add another to the well known list of horrible examples.

A recent conference in Pennsylvania on the potato-wart situation brings out a possibility that the limited infections of this disease in Pennsylvania, West Virginia, and Maryland may be effectively eliminated by eradication measures involving soil treatment with ammonium sulfocyanate. In connection with such a program your committee believes that eradication effort should be accompanied by extensive surveys of areas in all 3 States where wart is likely to occur.

Your committee has received a copy of the report of the special quarantine committee appointed at the summer meeting at Chicago and concurs in the views expressed in this report.

In accordance with the recommendation in the special committee's report urging concerted study in various countries of the plant pathological basis of quarantine problems, your committee believes that this society might well indicate to the officials in charge of arranging the coming International Botanical Congress in Amsterdam in 1935 our hope that some consideration of this subject may be included in the agenda. In this connection your committee believes that the relations of virus troubles, nematodes, and bacterial diseases to quarantine activities in general, are much in need of attention.

The question of merging the duties of this committee with those of the committee on Foreign Pests and Diseases has been carefully considered by members of both committees and it is recommended that this action be taken.

C. R. ORTON, *Chairman*,  
J. S. BOYCE,  
H. T. GÜSSOW,  
J. F. ADAMS,  
W. A. MCCUBBIN.

In accordance with the foregoing recommendations the Council has named the following as a committee on Foreign Plant Diseases: C. R. Orton, *Chairman*, H. T. Güssow, J. S. Boyce, W. A. McCubbin, R. D. Rands.

#### COMMITTEE ON EXTENSION WORK IN PLANT PATHOLOGY

The extension committee sponsored a barley-disease conference at Dubuque, Iowa, in September that was attended by pathologists, agronomists, and commercial interests of the surrounding States aided by representatives of the U. S. Department of Agriculture extension service, grain grading service, and cereal office. As a result of this conference barley grading schools are being arranged for Minnesota, Iowa, Wisconsin, and Illinois. Barley scab and *Helminthosporium* head blight were the diseases given major attention.

Arrangements have been made for the Boston meeting with Dr. Chupp as chairman. Topics for discussion include: (1) New seed disinfectants and fungicides; (2) commercial seed treatment, its advantages and limitations; (3) seed treatment and other service work in the countries; (4) emergency-garden relief work; (5) new points on illustrative material and its use.

The stencil circular "The Extension Pathologist" has been issued at irregular intervals under the direction of R. J. Haskell, Extension Service and Bureau of Plant Industry, cooperating. This circular has been helpful in the exchange of ideas, and will be continued.

Respectfully submitted,

R. E. VAUGHAN, *Chairman*.

#### COMMITTEE ON NECROLOGY

During the calendar year 1933, there have been two deaths, as follows:

Mr. Arthur Bliss Seymour, March 29, 1933.

Dr. James Abraham Faris, September 24, 1933.

It has been learned recently also that Mr. Harold Peirce died April 12, 1932.

A. G. JOHNSON, *Chairman*,

G. P. CLINTON,

M. B. WAITE.

#### REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

*Committee on Elections.* The committee on elections, after having opened sealed-ballot envelopes and counting ballots for each nominee, reports election of the following officers: President, N. E. Stevens, Vice-President, G. W. Keitt, Councilor, F. D. Heald.

A. N. BROOKS, *Chairman*,

H. S. CUNNINGHAM.

*Auditing Committee.* The committee appointed to audit the accounts of the Secretary-Treasurer and Business Manager of PHYTOPATHOLOGY has examined the books of this official and found them correct and in good order.

The committee commends the care with which these several accounts have been kept.

JOHN STEVENSON, *Chairman*,

JOHN W. ROBERTS.

*Report of Temporary Committee on Plant Quarantines Appointed at Chicago Meeting 1933.* The temporary committee on plant quarantines appointed at the Chicago meeting respectfully submits the following report.

We unanimously approve the suggestions of Dr. O. Appel in his Chicago paper regarding plant quarantines, namely, that the biological basis of plant quarantines is an appropriate subject of consideration by plant pathologists and that concerted world-wide study of the biological factors involved is desirable.

We feel that this Society has a definite responsibility to encourage the study and discussion of plant-disease problems in relation to quarantines.

We congratulate the authors of University of California Bulletin No. 553, "The Efficacy and Economic Effects of Plant Quarantines in California," on the discussion of the questions therein contained and commend this Bulletin to the consideration of our members.

We endorse as worthy of hearty support the present policy of the U. S. Quarantine authorities in frequently reviewing at public conferences questions of quarantine policy and procedure.

We respectfully request that this report be accepted and a copy filed with the standing committee on plant quarantines and that our temporary committee be discharged.

L. R. JONES, *Acting Chairman*,

J. DUFRÉNOY,

H. T. GÜSSOW,

H. H. WHETZEL,

E. C. STAKMAN,

W. A. McCUBBIN,

N. E. STEVENS.

*The Report of Representatives on the Council of the A. A. A. S.* The duties of Society Representatives on the Council of the A. A. A. S. are chiefly voting on candidates for offices and titles for Section "G" programs. We have consistently voted in such

matters in accordance with individual judgment. If members of this Society have suggestions as to desirable choices, we shall be glad to receive them.

NEIL E. STEVENS,  
H. S. CUNNINGHAM.

*Resolutions Committee.* The American Phytopathological Society wishes to express its thanks to the American Association for the Advancement of Science for making general arrangements for these meetings. It also is indebted to Dr. Sam Prescott of the Massachusetts Institute of Technology and his associates for arranging many of the details of the meetings.

The Society wishes to express its thanks to James Biggar, supervisor of Harvard Building arrangement facilities, to Thomas Lawlor and Paul Walker, supervisors of student aides for motion-picture projectors, lantern service, preparation of rooms, and handling of exhibits.

For the very efficient and courteous service to its members during these meetings, The American Phytopathological Society wishes to express its appreciation to the management of the Westminster Hotel. Special thanks are due to Mr. Joseph McCarthy, Manager, to Herbert Penney, who managed the banquet, and to Willard C. Stimpson, Andrew Anderson, John Olson, and Thomas Garvey.

Special applause and appreciation are extended to Director Thieelavia, Acetic Acid Doran, Toast Master Aloysius P. McGinty, The Big Bad Wolf of the Tobacco Belt and to Dr. Operculate Kitrid Sparrow, members of the expedition to McGinty Archipelago, for the mirth-provoking entertainment given members of The American Phytopathological Society and their friends who attended the annual banquet of the Society.

Deep and sincere appreciation are again extended to F. C. Meier, Secretary-Treasurer of the Society and Business Manager of PHYTOPATHOLOGY, and to H. B. Humphrey, Editor in Chief of PHYTOPATHOLOGY, and his associates for their continued, efficient services and for their constant contributions of time and energy in behalf of The American Phytopathological Society.

W. H. TISDALE,  
S. M. ZELLER,  
ANNIE R. GRAVATT.

#### ACTION OF THE COUNCIL

In addition to making the appointments of the officers mentioned earlier in this report, the Council submitted the following actions, which were approved by the Society:

1. It is recommended that, as was the case in 1933, the Editor in Chief of PHYTOPATHOLOGY be allowed actual expenses up to or within \$300 for secretarial and editorial assistance.
2. That the Editor in Chief of PHYTOPATHOLOGY be authorized to select the necessary associate editors for the year 1934.
3. It is recommended that the Secretary-Treasurer be authorized to contribute \$15.00 to defray expenses of the Biological Smoker in Boston.
4. It is recommended that in view of the long-established custom and the advantages derived from contacts with members of other professional groups, this Society continue the practice of meeting with the American Association for the Advancement of Science.
5. It is recommended that the Secretary-Treasurer be instructed to insert a one-page advertising notice in PHYTOPATHOLOGY for the purpose of ascertaining member personnel available for employment and opportunities available for work.



## OTHER BUSINESS

The reports of officers, committees, and representatives as printed above were accepted.

A motion made by I. E. Melhus that the Chair appoint a committee to place the following resolution before the appropriate officers of the Federal Government and to cooperate with them in formulating a satisfactory program was carried:

*Be it Resolved*, that The Phytopathological Society believes that a State and National serious hazard prevails in the impaired state of health of our tree population, which is in sore need of study as to cause, prevalence, distribution, and destructiveness, that Congressional Act—1105—73d Congress (S-598) provides for plant-disease control and for forest research, and that, to date, no adequate phytopathological program has been undertaken under this Act, and that the Society urges upon those charged with the responsibility of administering the Act to formulate such a program in cooperation with the several States.

**ARTHUR BLISS SEYMOUR**

JANUARY 3, 1859—MARCH 29, 1933

Arthur Bliss Seymour was graduated from the University of Illinois in 1881 with the degree of Bachelor of Science and, in 1886, he received the degree of Master of Science from the same institution.

From 1881 to 1883, and again for a period in 1884, he was botanist for the State Laboratory of Natural History for Illinois. From 1883 to 1885, except for the period in 1884 referred to above, he was private assistant to Dr. Farlow in the Cryptogamic Herbarium at Harvard University. From 1885 to 1886, he was instructor in botany at the University of Wisconsin. From 1886 to the time of his death he served as assistant in the Cryptogamic Herbarium of Harvard University.

Mr. Seymour served as editor of cryptogamic botany for a section (A-G) of the Century Dictionary in 1888, and from 1890 to the time of his death he served as chief editor for economic fungi for the same publication. He was the author or joint author of numerous publications chiefly on mycology and plant pathology, and was compiler of that stupendous and very valuable work "Host Index of the Fungi of North America." The painstaking care and persistence with which he followed up this work exemplifies his principal ideal in life, namely, "service for others." He contributed in many important ways to the fields of mycology and plant pathology.

**JAMES ABRAHAM FARIS**

JULY 27, 1890—SEPTEMBER 24, 1933

James Abraham Faris was graduated from the Northwest Missouri State Teachers College at Maryville in 1913. He received the degree of Bachelor of Science from the University of Missouri in 1916, his Master's degree from the University of Nebraska in 1920, and the degree of Doctor of Philosophy from Columbia University, New York City, in 1923.

During 1916-17 Dr. Faris was Professor of Botany and Biology at Stephens Junior College, Columbia, Missouri, and during 1917-18 he was Professor of Botany at the City Junior College, St. Joseph, Missouri. From 1918 to 1920, he was Pathologist in the Office of Cereal Investigations and employed as State leader in barberry eradication in Nebraska. From 1920 to 1921 he made investigations in Santo Domingo for the Dominican Government on diseases of economic crops of that country. From 1921 to 1924, he was Research Fellow of the National Research Council and the Brooklyn Botanic Garden, Brooklyn, N. Y. From 1924 to May, 1931, Dr. Faris was Chief Pathologist of the Tropical Plant Research Foundation and the Cuba Sugar Club and was stationed at Central Baraguá, Cuba, where he served also as Assistant Director of the Sugar Planters Experiment Station. On June 1, 1931, Dr. Faris was appointed Senior Pathologist in the Division of Cereal Crops and Diseases, in charge of investigations of cereal smuts, which position he held until the time of his death.

Dr. Faris made important contributions to the scientific knowledge of plant diseases and their control, particularly the smut diseases of cereal crops and the various diseases of sugar-cane. He was a man of unusual ability and integrity, combining keen, well-balanced judgment with aggressive leadership and personal charm.

# PHYTOPATHOLOGY

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## A COMPARATIVE STUDY OF CERTAIN TISSUES OF GIANT-HILL AND HEALTHY POTATO PLANTS<sup>1</sup>

HELEN DEUSS HILL<sup>2</sup>

(Accepted for publication July 5, 1933)

### REVIEW OF LITERATURE

Folsom (9) in 1926, reporting on viroses of the potato, classified them in 5 groups as follows: leaf roll, mosaic, aucuba mosaic, streak, and spindle tuber. The last-named group includes the malady known as giant hill. Though giant hill is thus classed as a virosis, Folsom (9) says it "has not yet been definitely proved to be transmissible from plant to plant" and that the diseases of this spindle-tuber group have not been tested for the presence of a pathogen with a known type of structure.

Mention of giant hill as a potato disease was first recorded in 1924 (24) as an "abnormal type of potato plant which Dr. E. L. Nixon discovered several years ago and to which he has given the apt name of Giant-Hill." The same year Folsom (8) also refers to the isolation of "another symptom aggregate . . . that breaks up tuber-units and is perpetuated in the tubers, and that seems to answer the typical description and some of the hill selection examples of Nixon's Giant-hill degeneration disease." In 1924 Gilbert (12), too, records a condition that has been "referred to as 'Giant Hill.'" Writing in 1925, Gilbert (13) called giant hill a "new phase of spindle tuber." In 1924, Bailey (4), in Vermont, and McCubbin, Hartman, and Lauer (19) in 1925 included giant hill among the serious diseases of the potato. Since then it has become recognized as a disease that, though not of equal importance in all potato-growing regions, is sufficiently serious to receive attention.

Since the gross appearance presented by giant-hill potato plants and certain tuber characteristics have been their chief distinguishing features, it may be well here to list some of them.

<sup>1</sup> Submitted in partial fulfillment for the Ph.D. degree. Contribution from the Department of Botany, The Pennsylvania State College, No. 94. Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, May 14, 1934, as Technical Paper No. 655.

<sup>2</sup> I am indebted to the members of the staff of the Department of Botany at The Pennsylvania State College and especially to Dr. E. L. Nixon and Dr. J. Ben Hill for helpful suggestions in the course of the investigation and in the preparation of the manuscript.

The following composite description of giant hill, comprising the essential characters presented by various authors (10, 12, 13, 18, 20), will suffice to acquaint the reader with the disease. The root system shows an abnormal fibrous development, with numerous and thicker roots as well, and the stolons are notably longer than in normal plants. The tubers, generally formed later, are of undesirable shapes for seed and market purposes, with constrictions, deep eyes, and heavy shoulders back of the eyes. One author (12) relates giant hill to spindle tuber on the basis of the similarity of their tuber shape. Usually there are one or two large, poorly shaped tubers and a number of very small ones.

Conflicting reports as to the effect on yields can perhaps be harmonized<sup>3</sup> by a consideration of environmental conditions. In Maine, Folsom (9) and Folsom *et al.* (11) report that the yield may be slightly less or as much as 20 per cent greater than normal. In Pennsylvania (19) giant hill is becoming an important limiting factor in commercial and seed crop production. Goss (14) and Goss and Peltier (15), as well as Nixon, believe that environment affects the symptoms, spread, and effect on yield of potato-degeneration diseases. Conditions adverse to good potato growth, such as drought, result in giant hill, in the building of vine and not tuber.<sup>3</sup> When conditions are favorable for potato growth, as they usually are in Michigan and Maine the giant hill characteristics are less marked.

Figures of total weights from 22 hills each in 1921 indicate the effect of giant hill on yield in Pennsylvania. Each hill had been planted from a separate potato, the seed pieces being equivalent in weight and comparably planted. Twenty-two hills of healthy plants from Lehigh County, produced 27.44 pounds of potatoes. Twenty-two hills of giant-hill plants from Cambria County produced 13.18 pounds, and the same number of giant hills from New York State, 14.63 pounds.

The aboveground portion of the giant hill plant generally is coarse, with excessive branching (20, 9), and vines taller than normal. (Fig. 1). During the early part of the growing season giant hill plants sometimes appear lighter green than healthy plants, but with the advancing season they become relatively darker and remain green and upright after other vines have died. The tendency of the vines to remain green and upright after other vines have died and to be resistant to the early frosts that kill most potato plants makes it possible at the end of the season to recognize giant hill and to rogue seed fields for the disease.

Giant-hill plants usually consist of but a single and very stiff stalk (31), of erect habit and an excessive amount of lateral growth proceeding from numerous axillary shoots (20). Nixon<sup>3</sup> refers to the "constriction of the

<sup>3</sup> Personal communication from E. L. Nixon, Professor of Plant Pathology, The Pennsylvania State College.

tops" of giant-hill plants, by which he means the more erect and compact habit, especially at the top of the plant as compared with the spreading nature of the healthy plants. The leaves are coarse with an irregular surface and pronounced purple tinge along the edges. In Rurals, the gloss and purple coloring of giant-hill vines is particularly noticeable.

According to Gilbert (12) the leaves are not rugose nor marginally inrolled, but Folsom (9) says rolling may be "slight or absent." Though slight chlorosis may be characterisite of the leaves (9) an account by Lauer (18) describes the foliage as dark green. A later and more prolonged blossoming period generally is observed in giant hill plants. In general, "all the characteristic symptoms of giant hill are those of excessive vegetative growth" (19).

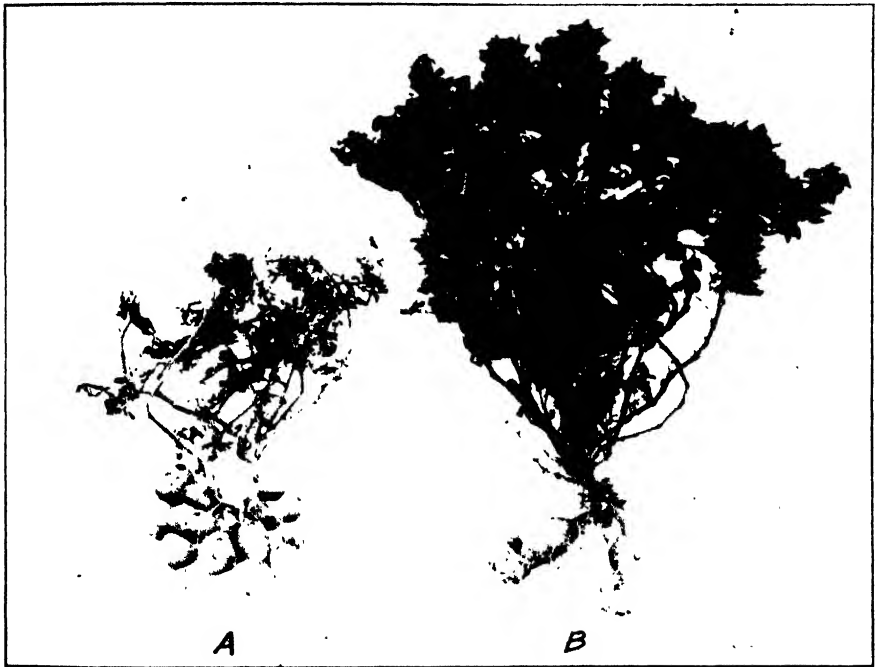


FIG. 1. A. Healthy plant with tubers. B. Giant-hill plant with tubers.

Artschwager (1), preliminary to the study of the pathological anatomy of plants suffering from various diseases, made a very comprehensive investigation of the anatomy of the potato plant. The bicollateral bundle, one in which there is phloem both outside and inside of the xylem, is one of the outstanding characteristics of the stem. Artschwager points out that the preponderance of sieve tubes in the secondary phloem makes the importance of the phloem in translocation appear self-evident. With the phloem occu-

pying such a strategic position in the healthy functioning of the potato plant, any disturbance in this tissue assumes a critical aspect.

Various investigations have shown histological relationships with diseased conditions. Quanjer (25) was the first to describe phloem necrosis in leaf-roll plants, a condition confirmed later by Artschwager (2). Disorganization in phloem, xylem, parenchyma, necrosis in cortex, collenchyma, and epidermis were reported also by Quanjer (22) in "streak." Other investigators (7) report a failure to find anatomical differences, but point out that potato phloem appears to be particularly sensitive in showing disorganization phenomena. Artschwager (3) emphasizes that it is the universality in distribution of changes in the phloem coupled with absence of necrosis in other tissues that gives such changes diagnostic value. Though von Brehmer (5) differentiated a "necrobiosis" in all potato plants with well developed phloem, he also mentions Quanjer's phloem necrosis. Phloem necrosis is regarded by Thung (32) as a symptom of leaf roll in a far advanced stage, with transport having been disturbed before the destruction became visible. Of a number of tuber-producing plants that put a heavy demand on food movement, curiously the phloem of the potato alone is subject to degeneration (30).

Some investigators have regarded the appearance of necrosis in phloem as an age phenomenon; but Rochlin (27) reports that, in mosaic-diseased potato plants, necrosis of the phloem occurs much earlier than in healthy plants, where it is found normally at maturity. In addition to various other changes, Rochlin reported necrosis of pith and cortical cells and shortening of the palisade and spongy parenchyma cells with almost complete absence of intercellular spaces.

These observations on the palisade cells confirm previous reports. Iwanowski (17), as early as 1903, had agreed with Woods (34) that palisade cells were shortened in tobacco leaves affected with mosaic and the intercellular space reduced. Melchers (21) failed to confirm Woods' observations on the absence of palisade parenchyma in the light colored areas of tomato leaves showing mosaic and its modification in moderately diseased plants; he did, however, report modifications of the palisade tissue in potato mosaic similar to those found by Woods in tomato and tobacco.

A very recent investigation by Verplancke (33) on spindle tuber, a degeneration disease classified by Folsom (9) with giant hill as one group of potato viroses, showed that spindle tuber had the effect of increasing the ratio of length to width of the cells of all the tissues of the tuber, or an increase in length and decrease in width over the cells of the healthy potato. He believes that the elongation of the tuber in spindle tuber is a specific result of the action of the causative virus.

Reports of previous investigations on the histological and anatomical

effects of various degeneration diseases of plants, though not altogether in agreement, seem to have shown definite changes principally in the palisade tissues of the leaves and in the phloem of the stem. These changes have involved more or less disorganization of the tissues concerned.

#### METHODS

Since the present study was undertaken to find out whether the giant-hill plants had any anatomical or histological characteristics that might be correlated with this fairly distinctive picture presented in the field, sections were secured of leaves, petioles, and stems of both giant-hill and healthy potatoes. In 1927, E. L. Nixon brought in samples from various potato fields in Pennsylvania. A small number of plants from his plot was studied in 1928. For the investigation of material collected in 1930, samples were taken from plants in one of the experimental plots on the college campus. These plants were grown from tubers from giant-hill and healthy plants secured by E. L. Nixon and L. T. Denniston from various fields in the State. The seed pieces were planted in alternate rows of giant-hill and healthy tubers. The plants contributing samples to this study were checked by Nixon for their continuing giant-hill or healthy character at 3 successive times during the growing season and again at harvest. The samples from giant-hill and healthy plants were secured at the same time, thus insuring their equivalent age. For the 1932 study, samples were secured both from plants on one of the experimental plots on the college campus and from a plot in Potter County. Precautions similar to those in 1930 were observed as to the identity of the plants.

The material studied in 1927 consisted of stems, petioles, and leaves. The plant portions were fixed in an acetic acid-formalin-alcohol solution, and imbedded in paraffin, following the usual alcohol-xylol series. The 1930 material was mainly secured in July and was similarly fixed, but was imbedded in paraffin following treatment with an ethyl alcohol-butyl alcohol series (Zirkle 35). The harder pieces of stem from the lower parts of the plants were treated with hydrofluoric acid after fixing and some of them were imbedded in celloidin instead of paraffin. The 1932 material was secured in early September and was treated like that obtained in 1930.

Leaf sections were cut 10–12 $\mu$ , petiole and stem sections, due to their hardness, from 15–22 $\mu$  thick. The safranin-light green stain was used for all sections cut from material imbedded in paraffin and iron alum-haematoxylin was used to stain sections cut from material imbedded in celloidin. Somewhat more than 3800 slides were examined in the study.

In 1927 micrometric measurements were made of (1) entire cross sections of leaves, thickness of epidermis, collenchyma, outer phloem, inner phloem and xylem in the widest region of petioles and stems; (2) length

and width of palisade cells from leaf cross sections, of diameter of palisade cells from leaf sections cut parallel to the surface; and (3) diameter in cross section of parenchyma cells of stems.

In 1930, the measurements were confined to entire cross sections of leaves, of outer and inner phloem and xylem where it was widest in cross sections of petioles and stems, of pith and entire stem diameters, and of length, width, and diameter of palisade mesophyll cells in both giant-hill and healthy plants.

In 1932, measurements were made of entire cross sections of leaves, of length and width of palisade mesophyll cells, and of outer and inner phloem and xylem of stems.

Because of the time involved in securing sections, the study was restricted to a few sections of a comparatively small number of plants. Though hundreds of measurements were made of the material secured in 1927, these were regarded merely as indications, which were used as a foundation for the study of the 1930 material, and are not here presented. The statistical study, the results of which are given here, includes the material secured in 1930 and in 1932. This restriction was justified by the carefully repeated checking as to characteristics of the diseased or healthy condition of the plants studied in 1930 and 1932 compared with the single judgment used on the samples secured in 1927.

Of the leaves, as many as 50 measurements were made of a single leaf or 50 measurements of palisade cells from a single leaf. In the later material only 10 measurements were made. In cross sections of petioles and stems 10 measurements per plant were made. Tables were arranged from these measurements, and the means and other necessary calculations were based upon them. Odds were calculated from the table of Pearl and Miner (23). In 1930, 17 healthy and 31 giant-hill plants were the basis of the statistical study and in 1932, 14 healthy and 16 giant-hill plants were studied.

## RESULTS

The tables<sup>4</sup> made of some of the measurements secured show that the material is highly variable, and calculations of the coefficients of variation confirm this indication. The percentages of variation are about the same for similar tissues in both the diseased and healthy plants. Determinations of probable errors and odds show, however, that, notwithstanding the variability, there are a few fairly consistent differences in some of the tissues of the giant-hill and healthy plants sampled. For the sake of greater accuracy, calculations were made in terms of micrometric measurements. The means have been translated to millimeters.

<sup>4</sup> Available for consultation on application to the Department of Botany, The Pennsylvania State College.



Leaf thickness was regarded in 1927 as indicating promising differences.

Measurements of both the 1930 and 1932 material showed that the leaves of the healthy plants were thicker than those of the diseased. A more careful analysis of the 1930 material (31 plants) showed that the mean

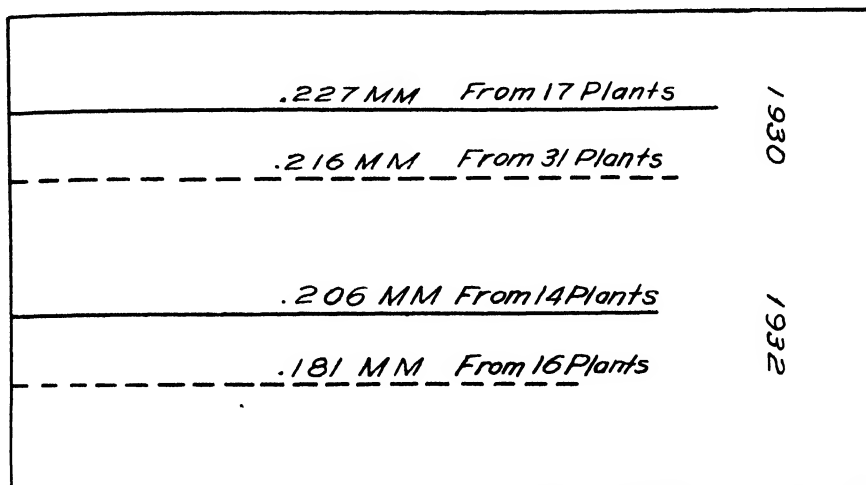


FIG. 2. Comparative measurements of leaf thickness expressed in means. Solid line for healthy, broken line for giant-hill plants.

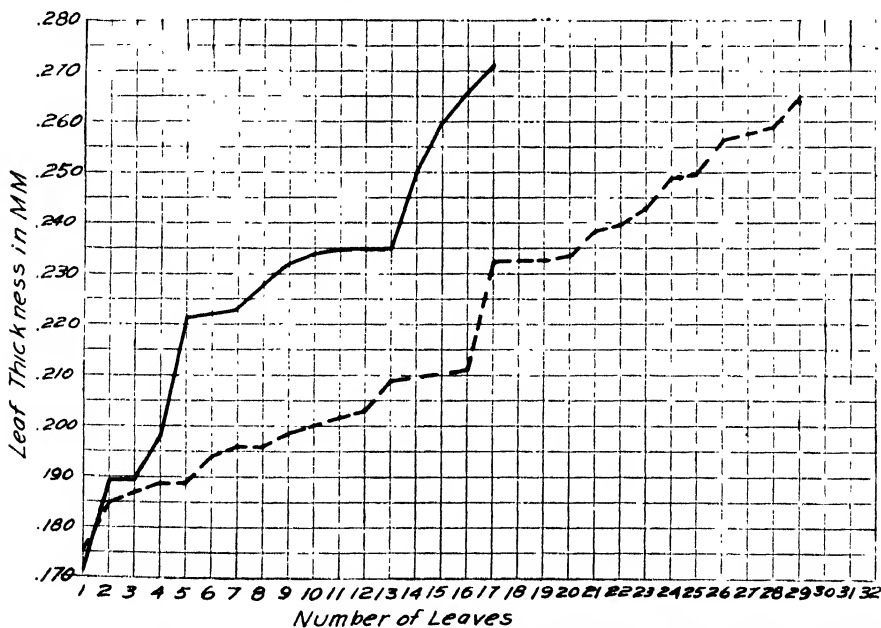


FIG. 3. Distribution of measurements of leaf thickness. Solid line for healthy, broken line for giant-hill plants.

of the thickness of the giant-hill leaves is 0.21665 mm. and that of the healthy (17 plants) is 0.22785 mm. In terms of micrometer spaces the mean thickness of the giant hill leaves is  $12.38 \pm 0.063$  and of the healthy,  $13.02 \pm 0.88$ , showing a difference of  $0.64 \pm 0.10$ . The ratio of 0.64 to 0.10 is high enough to indicate odds of nearly 20,000 to 1 that this difference is a significant one. If the mean thickness of the healthy leaves for 1930 be represented by 100, that of the leaves from the giant-hill plants will be as 95.08. For further comparison see figures 2 and 3. For the 1932 material, if the mean thickness of the healthy be represented by 100, that of the leaves from the giant-hill will be as 87.

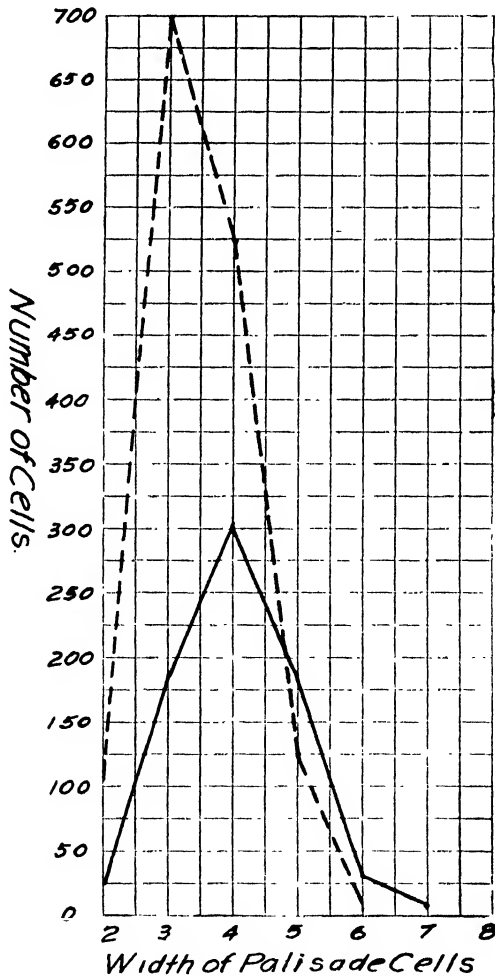


FIG. 4. Distribution of measurements of width of palisade cells, in terms of micrometer spaces. One space = 0.004 mm. Solid line for healthy, broken line for giant-hill plants.

The analysis of the material secured and studied in 1932 confirms these results, but with even greater statistical significance. In this case the mean of the thickness of giant-hill leaves (16 plants) is 0.181 mm. and that of the healthy (14 plants) is 0.206 mm. In terms of the spaces used, the mean

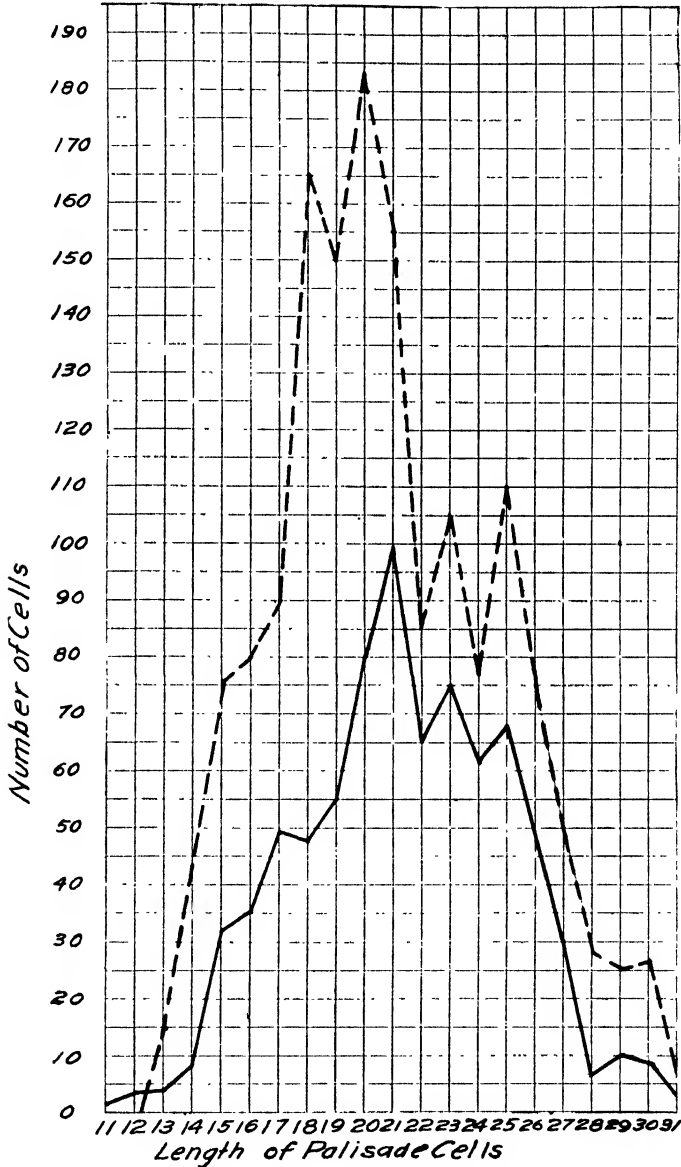


FIG. 5. Distribution of measurements of length of palisade cells, in terms of micrometer spaces. One space = 0.004 mm. Solid line for healthy, broken line for giant-hill plants.

of the giant-hill is  $10.38 \pm 0.134$  and of the healthy  $11.93 \pm 0.047$ , showing a difference of  $1.55 \pm 0.14$ . The ratio of the difference to its probable error indicates that this trial would need to be repeated more than 1,000,000 times before a difference as great as this, attributable to chance alone, could be found. In view of the fact that these differences in leaf thickness in giant-hill and healthy potato plants are in the same direction and of so great a magnitude in relation to the total leaf thickness, it can be safely affirmed that leaves of giant-hill potato plants are definitely thinner than those of healthy plants.

This difference in leaf thickness led to the suggestion that the palisade mesophyll tissue might also show differences. A study of the cells of the palisade mesophyll tissue from 1930 material confirmed the first impression of such differences. Graphs made of lengths, widths, and ratios of widths to lengths of these cells (Figs. 4, 5, 6) indicated that they showed differ-

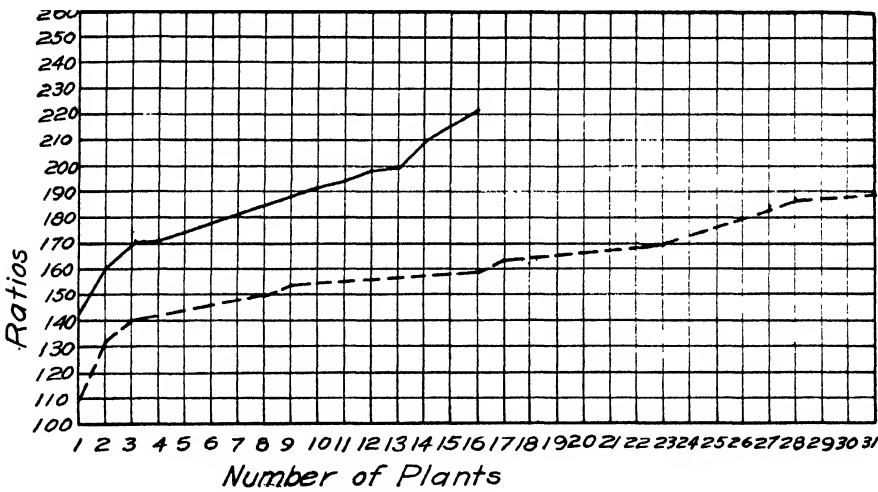


FIG. 6. Distribution of ratios of width to length of palisade cells. Solid lines for healthy, broken line for giant-hill plants.

ences in the diseased and healthy condition. The means of the widths were found to be 0.01584 mm. for the giant-hill and 0.01748 mm. for the healthy; of the lengths 0.085604 mm. for the giant-hill and 0.08644 mm. for the healthy. In terms of spaces these show the following :

Cell dimension	Giant-hill	Healthy	Giant-hill less than healthy by these amounts
Mean width . . . . .	$3.96 \pm 0.0123$	$4.37 \pm 0.021$	$0.41 \pm 0.0243$
Mean length . . . . .	$21.401 \pm 0.0067$	$21.61 \pm 0.00947$	$0.21 \pm 0.011$
Width-length . . . . .	$0.37 \pm 0.0068$	$0.64 \pm 0.0029$	$0.27 \pm 0.00007$

Measurements of lengths and widths of palisade mesophyll cells (Fig. 7) from the 1932 material show differences between giant-hill and healthy plants in the same direction. The means of the widths were found to be 0.01292 mm. for the giant hill and 0.01512 mm. for the healthy plants; of the lengths 0.07140 mm. for the giant hill and 0.08052 mm. for the healthy. In terms of spaces these show the following:

Cell dimension	Giant-hill	Healthy	Giant-hill less than healthy by these amounts
Mean width .. . . .	$3.23 \pm 0.04$	$3.78 \pm 0.05$	$0.55 \pm 0.06$
Mean length . . . . .	$17.75 \pm 0.23$	$20.13 \pm 0.22$	$2.38 \pm 0.31$

On the basis of these figures from both the 1930 and the 1932 material, it can be stated with reasonable assurance that the palisade mesophyll cells of the giant-hill plants are considerably narrower and somewhat shorter than those of the healthy.

It is evident from a comparison of the figures for 1930 and 1932 that the latter show the greater differences between giant-hill and healthy leaves, both as to their thickness and the length and width of their palisade cells. 1932 was a year showing even more disastrous effects on potato plants than 1930. These comparisons of diseased and healthy leaves furnish added confirmation for the belief rather generally held that adverse growing conditions emphasize diversities that might normally be less apparent.

An analysis of the relation of diameter and length of palisade cells of the 1930 material also revealed some differences. Since it was impossible to secure the diameter and length of one and the same cell as can be done for the dimensions length and width it was necessary in the calculations to use averages of measurements per leaf and thus to sacrifice some of the apparently greater significance which can attach to results based on the larger numbers from individual cell measurements.

Using the 1930 data, the correlations between lengths and diameter were found to be: Giant-hill plants,  $0.55 \pm 0.023$ ; healthy plants,  $0.28 \pm 0.087$ . Difference,  $0.27 \pm 0.089$ .

In the calculations of the means of the diameters from averages per leaf the differences between those of giant-hill and healthy plants were relatively large, the mean of the giant-hill plants being 0.01612 mm. and that of the healthy 0.01824 mm.

Expressed in terms of micrometer spaces the measurements are as follows:

Cell dimension	Giant-hill	Healthy	Difference
Mean diameter .....	4.07 ± 0.042	4.50 ± 0.098	0.51 ± 0.01

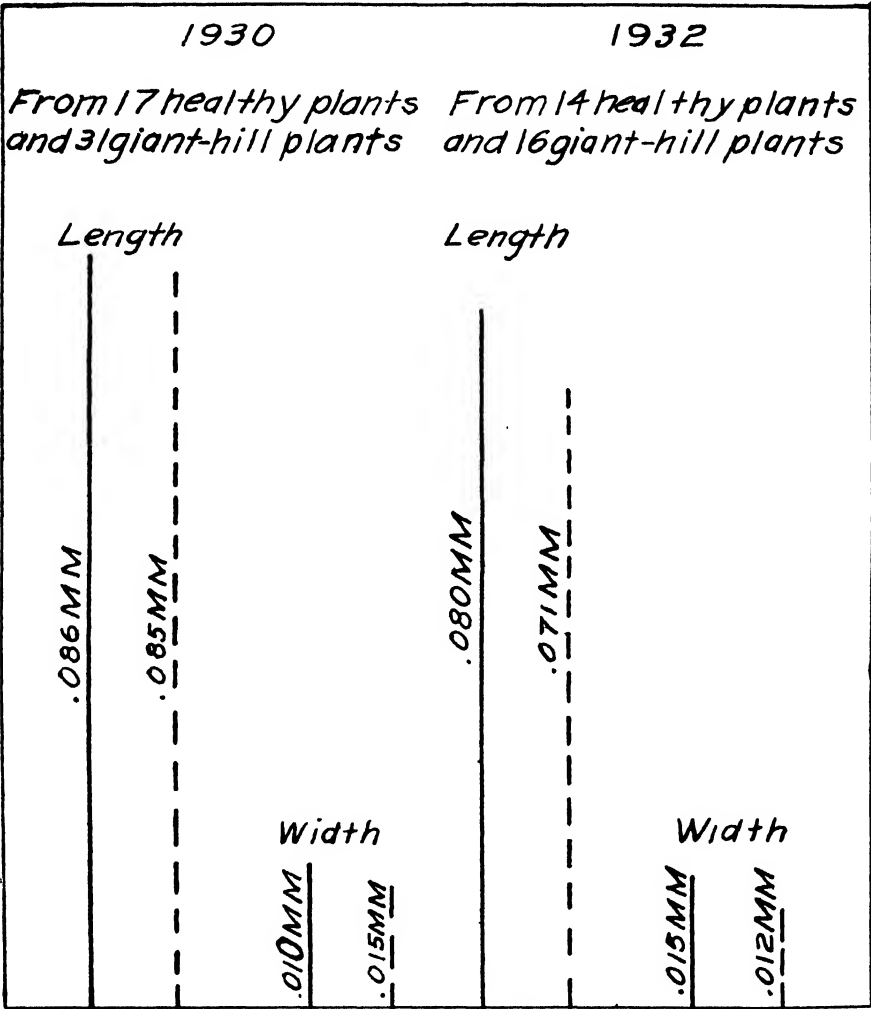


FIG. 7. Comparative measurements of cells of palisade mesophyll expressed in means. Solid line for healthy, broken line for giant-hill plants.

For further comparisons see figure 7.

In the consideration of the differences between the palisade mesophyll cells of the giant-hill and the healthy potato it would appear that those of the giant-hill have a smaller ratio of width to length than the healthy. The

diameters of the giant-hill mesophyll cells are also smaller than the healthy. These differences are indicated further in a comparison of the volumes of these cells calculated on the basis of averages per leaf. Palisade cells from thirty-one plants of giant hill showed a range of volume from 0.0001066 cu. mm. to 0.0002838 cu. mm., with a mean of 0.0001589 cu. mm.; the range for sixteen healthy plants is from 0.0001360 cu. mm. to 0.0003130 cu. mm. with the mean at 0.0002170 cu. mm.

In terms of micrometer spaces the volume calculations for palisade cells are as follows:

Cell dimension	Giant-hill	Healthy	Difference
Mean	248.38 $\pm$ 16.34	337.5 $\pm$ 22.58	89.12 $\pm$ 26.73

The odds are greater than 40 to 1 that this difference is significant. The difference may be otherwise expressed; if the mean volume of palisade cells of healthy leaves be represented by 100, that of giant-hill would be as 73.59.

Counts made of the number of cells to 0.2 mm., the length of the micrometer scale, indicated that there was no significant difference in the number of palisade mesophyll cells per 0.2 mm. in leaves of giant-hill and healthy plants. Since the palisade cells in the leaves of healthy plants were found larger than those of giant-hill, this lack of significant difference in cell number needs an explanation. Other things being equal, one would expect to find a larger number of cells associated with the smaller size in the giant-hill plants. Observation, however, indicates that there is slightly more intercellular space in the palisade region in the leaves of giant-hill than in those of healthy plants, and this harmonizes with the lack of difference in the number of cells. The appearance of the chloroplasts shows no great and uniform difference in the cells of the plants under comparison, though there may be an indication that those in the palisade cells of the giant-hill are somewhat less plump and round than those of the healthy leaves. It is of interest to note that the starch grains are associated with the chloroplasts, either actually within the chloroplasts or on their surface.

A comparison of measurements and leaf counts of 9 healthy and 7 giant-hill plants reveals differences that harmonize with field descriptions of these plants. The top portions of these plants, including 5 nodes with their attached leaves, show a mean of 32.0  $\pm$  1.3 cm. total length for the healthy and 25.28  $\pm$  0.51 cm. for the giant-hill, a difference of 6.72  $\pm$  1.39. This is a definite expression of the "constriction of the tops" observed above. The tips of these same stems, on the other hand, showed a mean of 9.0  $\pm$  0.62 leaves for the healthy and 4.85  $\pm$  0.41 for the giant-hill plants, a difference of 4.15  $\pm$  0.74.

The leaves of giant-hill plants show comparatively few macroscopic features comparable to those of potato plants affected with various other viroses. Folsom *et al.* (11), however, mention slight chlorosis as a characteristic, while Lauer (18) emphasizes the dark green of the leaves. Several writers (6), (31), refer to slight rolling of the margins. In studies of tomato and tobacco leaves (26), (27), and potato leaves (28) affected with mosaic, various modifications of the palisade tissue have been reported. Salaman and Bawden (29) state that it is "probable that pathological conditions of the leaf lead to phloem necrosis." In view of this suggestion, it seems pertinent to add the further suggestion that in giant hill plants, where the leaves show definite histological differences from the normal and where there is certainly some disturbance of the phloem relations in the stem, we have the possibility of a relationship between these two features in leaves and stems. Potato plants affected with giant hill, in regions where the disease is important, show a reduced storage of carbohydrates in their lessened tuber production. It is perhaps not too presumptuous to suggest that there may be a relation between this reduction of carbohydrates in the tubers and the disturbance in palisade mesophyll cells, which is indicated in the analysis of their measurements here presented.

A careful study of the leaf tissues showed no such cell inclusions as are reported by Hoggan (16) to be invariably present in the chlorotic areas of tobacco leaves affected with mosaic. The technique employed may have failed to reveal them. But Hoggan also reports the absence of such inclusions in the cases of 8 other viroses.

The measurements of the petioles of giant-hill and healthy plants from 1930 material show that in the former the inner phloem is slightly more extensive than in the healthy plants, but not significantly so. The xylem also appears to be more extensive in the petioles of giant-hill than in those of healthy plants. The outer phloem, on the other hand, is somewhat less extensive in the former than in the latter. The mean of the xylem in petioles of giant-hill plants is 0.1614 mm. and of the xylem of healthy plants is 0.1389 mm. The mean of the inner phloem is 0.1604 mm. in giant-hill and 0.1508 mm. in the healthy plants. Expressed in micrometer spaces these measurements are as follows:

Thickness in cross section	Giant-hill	Healthy	Increment Giant-hill over healthy
Mean xylem . . . . .	9.20 $\pm$ 0.11	8.34 $\pm$ .010	0.85 $\pm$ 0.15
Mean inner phloem . . . . .	9.23 $\pm$ 0.18	8.62 $\pm$ 0.17	0.61 $\pm$ 0.25
$\gamma$ Xylem and phloem . . . . .	0.45 $\pm$ 0.016	0.28 $\pm$ 0.0097	0.16 $\pm$ 0.018



The comparison of the correlations between xylem and inner phloem in the petioles shows that both the xylem and the inner phloem appear to be slightly greater in amount in the giant-hill than in the healthy plants and that the correlation between xylem and phloem is significantly higher in the diseased than in the healthy plants.

If the inner phloem in the petioles of healthy plants be represented by 100, that in petioles of giant-hill plants would be as 107.07. The odds, however, are very low that this is a significant difference. If the xylem in the petioles of healthy plants be represented by 100, that in petioles of giant-hill plants would be as 116. If the outer phloem in the petioles of healthy plants be represented by 100, that in petioles of giant-hill plants would be as 94.5. For further comparisons, see figure 8.

This situation in the petioles is of particular interest in connection with

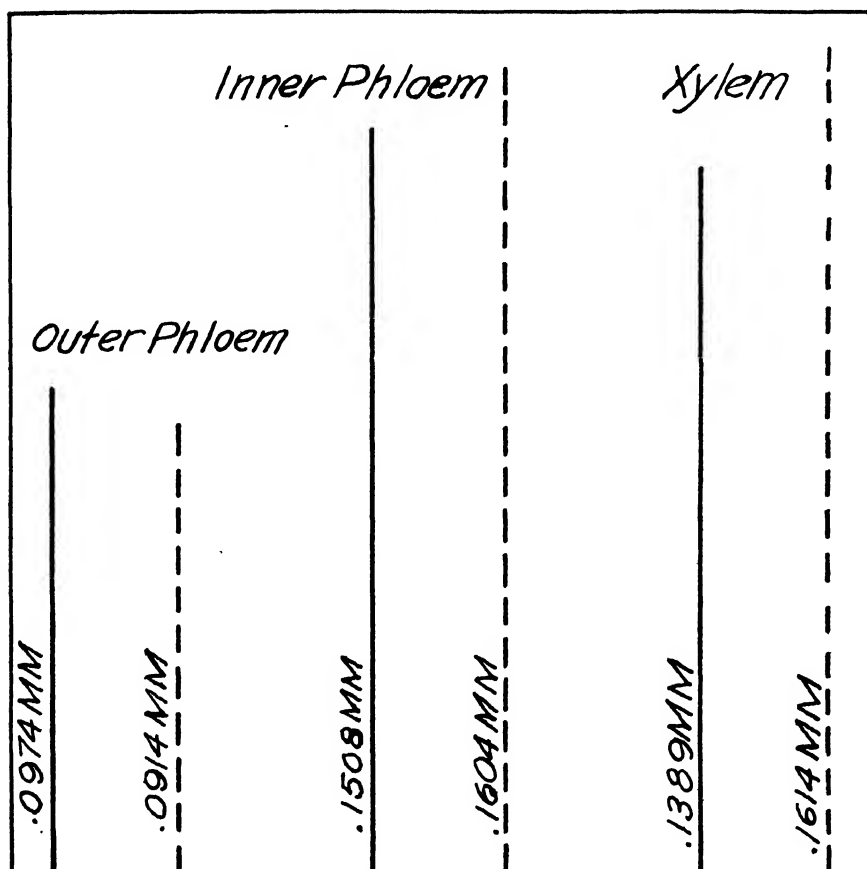


FIG. 8. Comparative measurements of xylem and phloem in petioles. Solid line for healthy, broken line for giant-hill plants.

that found in the stems. Similar comparative measurements in stems show that the inner phloem in the stems of healthy plants is significantly of greater extent than in the giant-hill plants. Computed for the 1930 material, the means of the outer phloem are 0.1240 mm. in the giant-hill and 0.1345 mm. in the healthy; of the xylem, 0.3046 mm. in the giant-hill and 0.3178 mm. in the healthy; of the inner phloem 0.3666 mm. in the giant-hill and 0.4782 mm. in the healthy. The measurements were made on 17 healthy and on 31 giant-hill plants.

In terms of micrometer spaces these tissues are measured as follows:

	Giant-hill	Healthy	Increment giant-hill over healthy
Mean outer phloem . . . . .	7.08 $\pm$ 0.92	7.74 $\pm$ 0.15	- 0.65 $\pm$ 0.93
Mean xylem . . . . .	17.41 $\pm$ 0.26	18.16 $\pm$ 0.26	- 0.75 $\pm$ 0.37
$\gamma$ Outer phloem and xylem	0.20 $\pm$ 0.036	0.15 $\pm$ 0.051	0.09 $\pm$ 0.06
Mean inner phloem	20.95 $\pm$ 0.28	27.33 $\pm$ 0.42	- 6.38 $\pm$ 0.504
Mean xylem . . . . .	17.39 $\pm$ 0.26	18.38 $\pm$ 0.22	- 0.99 $\pm$ 0.89
$\gamma$ Inner phloem and xylem	0.18 $\pm$ 0.038	0.49 $\pm$ 0.01	0.30 $\pm$ 0.042

Not only is the inner phloem of the healthy stems of greater extent than that of the giant hill, but the xylem also is very slightly greater and the correlation with the xylem, too, is greater.

If the inner phloem in the stems of healthy plants be represented by 100, that in stems of giant hill plants would be as 76.65. The odds that this difference is significant are greater than 20,000 to 1. For further comparisons see figure 9.

The analysis of measurements made of xylem and phloem in stem sections from the 1932 material shows similarly that the xylem and both the inner and outer phloem, as measured radially, are less extensive in the giant-hill than in the healthy plants. In 1932, stem measurements were made from 2 comparable regions, the second and third internodes from the top. For the second internode, the means of the outer phloem are 0.07995 mm. for the giant-hill and 0.085225 mm. for the healthy; of the xylem, 0.235550 mm. for the giant-hill and 0.280900 mm. for the healthy; of the inner phloem 0.258475 mm. for the giant-hill, and 0.3165 mm. for the healthy. For the third internode, the means of the outer phloem are 0.096775 mm. for the giant-hill and 0.1001 mm. for the healthy; of the xylem 0.3787 mm. for the giant-hill and 0.387275 for the healthy; of the inner phloem, 0.3556 mm. for the giant-hill and 0.403725 mm. for the healthy plants.

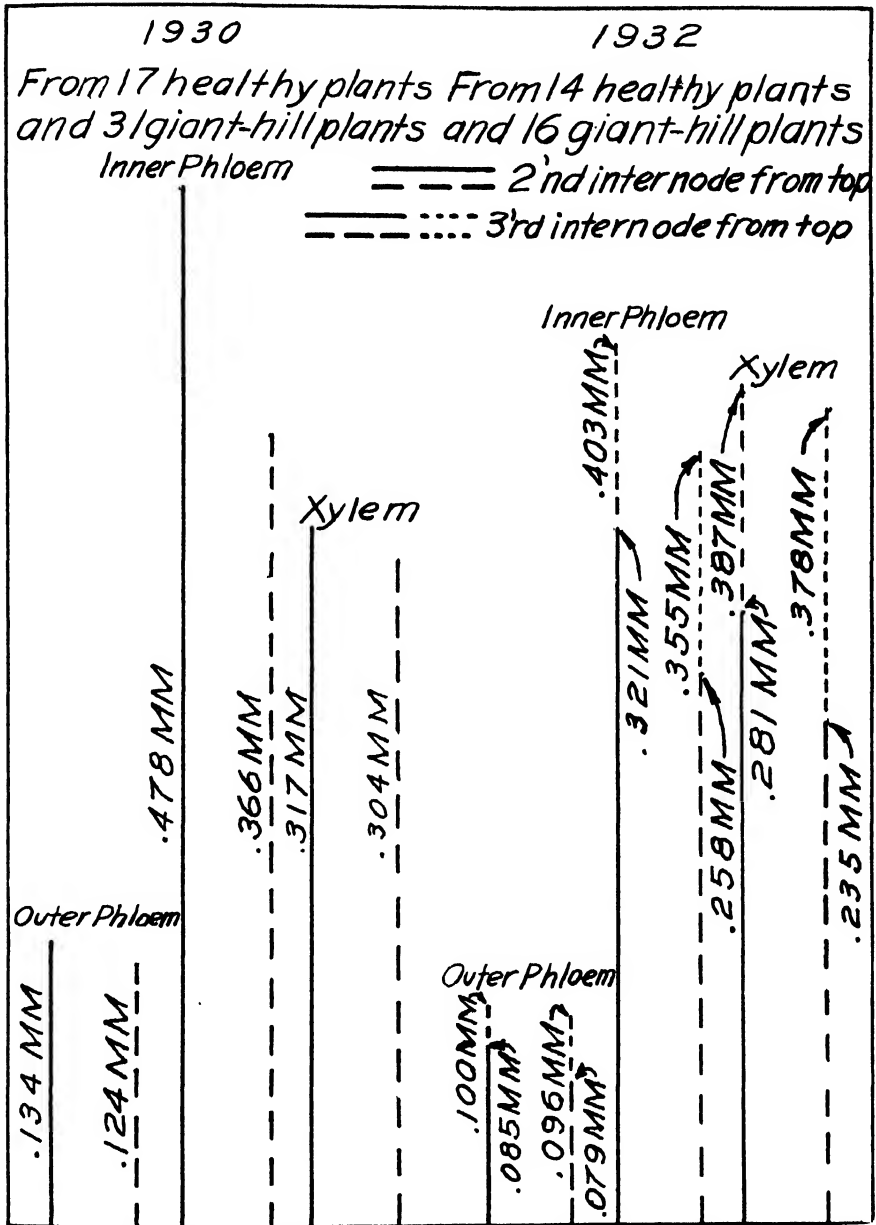


FIG. 9. Comparative measurements of xylem and phloem in stems in cross section expressed in means. Solid line for healthy, broken line for giant-hill plants.

In terms of micrometer spaces, these tissues in the 1932 material show the following measurements:

## Second Internode from the Top

	Giant-hill	Healthy	Increment giant-hill over healthy
Mean outer phloem ...	$4.74 \pm 0.09$	$4.87 \pm 0.07$	$-0.13 \pm 0.11$
Mean xylem . . . . .	$13.46 \pm 0.56$	$16.04 \pm 0.42$	$-2.58 \pm 0.7$ (odds 60 + to 1)
Mean inner phloem ..	$14.77 \pm 0.28$	$18.38 \pm 0.38$	$-3.61 \pm 0.47$ (odds over 400,000 to 1)

## Third Internode from the Top

	Giant-hill	Healthy	Increment giant-hill over healthy
Mean outer phloem ..	$5.53 \pm 0.10$	$5.72 \pm 0.13$	$-0.19 \pm 0.16$
Mean xylem . . . .	$21.64 \pm 0.52$	$22.13 \pm 0.53$	$-0.49 \pm 0.74$
Mean inner phloem ..	$20.32 \pm 0.31$	$23.07 \pm 0.65$	$-2.75 \pm 0.72$ (odds 95 + to 1)

The differences shown, particularly by the inner phloem in the 1930 material, are again evident in the 1932 material by odds well above those considered necessary for reasonable assurance. The differences in the outer phloem are statistically insignificant and the xylem shows significant differences in the second but not in the third internode. It is possible that the greater differences shown by the phloem from the second internode from the top over those from the third may be related to the more striking differences in macroscopic features exhibited at the tops of the plants.

Both the petioles and the stems of the giant-hill plants thus show differences from the healthy. The present investigation shows a slightly increased amount of phloem in the petioles or giant-hill plants over that in the normal and a greatly decreased amount of phloem in the stems.

Studies on related viroses (25), (27), (17), (2), (3) have shown various disorganization effects both in the palisade mesophyll of the leaves and in the phloem, especially of the stem. In the case of most of these other diseases the disastrous effects on the host plants are much more readily seen than in giant hill. It is of interest in this connection that giant hill, though causing less conspicuously harmful effects on the potato plants than some of the related viroses, appears to show histological effects, which, though also less conspicuous, are nevertheless similar in their nature to those of these other viroses. The cell activity concerned in photosynthesis must be disturbed where the tissues involved are reduced, as is the case in the leaves in giant-hill plants. And the conducting tissues, though showing no easily observed necrotic modifications, as reported in some of the studies

mentioned above, exhibit variations that must doubtless have associated with them certain physiological disturbances.

An intensive study of the cellular elements comprising the phloem and xylem failed to show any differences between the giant-hill and the healthy plants in these tissues. In view of this absence of differences in the cells it is particularly interesting to note tendencies toward disturbances in the amounts of these tissues present. Studies such as those of Artschwager (2, 3) and Thung (32) of the vascular tissues in stems of potatoes affected with supposedly related diseases show rather definite disorganization of the phloem. Perhaps we have here in giant hill, regarded by Nixon<sup>5</sup> as the precursor of leaf roll, a condition in which phloem disturbance, though not advanced to the stages of necrosis observed in the striking cases of leaf roll, is, nevertheless, present to the extent of showing changes from the healthy condition.

TABLE 1.—*Summary of difference between giant-hill and healthy-potato plants*  
Cells of Palisade Mesophyll

1930					
	Length	Width	Diameter	Ratio W.L.	Volume
Healthy . . . . .	21.61 $\pm$ 0.009	4.37 $\pm$ 0.021	4.58 $\pm$ 0.098	0.182 $\pm$ 0.003	337.5 $\pm$ 22.58
Giant hill ....	21.4 $\pm$ 0.006	3.96 $\pm$ 0.01	4.07 $\pm$ 0.042	0.156 $\pm$ 0.002	248.38 $\pm$ 10.29
Difference . . . . .	0.21 $\pm$ 0.01	0.41 $\pm$ 0.02	0.51 $\pm$ 0.01	0.026 $\pm$ 0.0036	89.12 $\pm$ 26.73
	odds greater than 1,000,000 to 1			odds nearly 500,000 to 1	odds greater than 40 to 1
1932					
	Length	Width			
Healthy . . . . .	20.13 $\pm$ 0.22	3.78 $\pm$ 0.05			
Giant-hill . . . . .	17.75 $\pm$ 0.23	3.23 $\pm$ 0.04			
Difference . . . . .	2.38 $\pm$ 0.31	0.55 $\pm$ 0.06			
	odds over 400,000 to 1		odds greatly over 1,000,000 to 1		

Measurements made under high power, expressed in terms of micrometer spaces.

1 space = 0.004 mm.

Leaf Thickness			
Mean		1930	1932
	Healthy . . . . .	13.02 $\pm$ 0.08	11.93 $\pm$ 0.04
	Giant-hill . . . . .	12.38 $\pm$ 0.06	10.38 $\pm$ 0.13
	Difference . . . . .	0.64 $\pm$ 0.10	1.55 $\pm$ 0.14
		odds nearly 20,000 to 1	odds greatly over 1,000,000 to 1

Measurements made under low power, expressed in terms of micrometer spaces.

1 space = 0.0175 mm.

<sup>5</sup> *Loc. cit.*

Tissues in Petioles				
Mean	Healthy .....	Outer phloem $5.62 \pm 0.07$	Inner phloem $8.62 \pm 0.17$	Xylem $7.93 \pm 0.07$
	Giant-hill ... ..	$5.22 \pm 0.06$	$9.23 \pm 0.18$	$9.19 \pm 0.11$
	Difference .....	$0.40 \pm 0.09$ odds about 150 to 1	$-0.61 \pm 0.25$ odds about 9 to 1	$-1.26 \pm 0.13$ odds greater than 1,000,000 to 1
Tissues in Stems				
1930				
Mean	Healthy .....	Outer phloem $7.74 \pm 0.15$	Inner phloem $27.33 \pm 0.42$	Xylem $18.16 \pm 0.26$
	Giant-hill ... ..	$7.08 \pm 0.92$	$20.95 \pm 0.28$	$17.41 \pm 0.26$
	Difference ...	$0.66 \pm 0.93$ insignificant	$6.38 \pm 0.50$ odds vastly over 1,000,000 to 1	$0.75 \pm 0.37$ insignificant
1932				
Second Internode from Top				
Mean	Healthy .....	Outer phloem $4.87 \pm 0.07$	Inner phloem $18.38 \pm 0.38$	Xylem $16.04 \pm 0.42$
	Giant-hill ... ..	$4.74 \pm 0.09$	$14.77 \pm 0.28$	$13.46 \pm 0.56$
	Difference ...	$0.13 \pm 0.11$ practically no significance	$3.61 \pm 0.47$ odds over 400,000 to 1	$2.58 \pm 0.70$ odds 64.79 to 1
Third Internode from Top				
Mean	Healthy ... ..	Outer phloem $5.72 \pm 0.13$	Inner phloem $23.07 \pm 0.65$	Xylem $22.13 \pm 0.53$
	Giant-hill . . .	$5.53 \pm 0.10$	$20.32 \pm 0.31$	$21.64 \pm 0.52$
	Difference .....	$0.19 \pm 0.16$ practically no significance	$2.75 \pm 0.72$ odds 95.15 to 1	$0.49 \pm 0.74$ no significance

Measurements made under low power, expressed in terms of micrometer spaces.  
1 space = 0.0175 mm.

#### SUMMARY

Measurements of stem lengths and leaf counts confirm the field description attributing greater constriction of the tops to giant-hill than to healthy potato plants.

The leaves of potato plants affected with giant hill were found to be thinner than those of the healthy plants.

The cells of the palisade mesophyll of leaves of potato plants affected with giant hill were smaller than those of the healthy plants, with a smaller ratio of width to length and smaller volume.

The inner phloem in the petioles of giant-hill plants was found to be slightly greater than that of the petioles of healthy plants and somewhat greater in relation to the xylem than in the healthy plants. The outer phloem was found to be somewhat less in the giant-hill plants.

The inner phloem in the stems of giant-hill plants was found to be considerably less than in the healthy plants and less extensive in relation to the xylem than in the healthy stems.

It is suggested that these disorganizations in photosynthetic and conducting tissues of the giant-hill plants are of related order though of less degree than the disorganizations reported for plants affected with some other virus diseases.

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# CYTOLOGY OF PLANT TISSUES AFFECTED BY VIROSES

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Few viruses cause local necrotic lesions. Few even cause the cells to die where they are inoculated. Most viruses when inoculated into a leaf do not develop any evident response at the inoculation point; neither does the inoculation induce evident changes in already differentiated tissue. But all tissues that later develop from a growing point of the inoculated plant may show cytological (12, 29, 30, 31, 33, 34, 37, 38, 39, 43, 46), histological, and even anatomical (4, 6, 24, 35, 42, 44) disturbances. Viruses, therefore, should first be considered as interfering with the growth and differentiation of embryonic tissues.

## DIFFERENTIATION OF EMBRYONIC TISSUES IN HEALTHY AND VIRUS-INFECTED BUDS

The actively dividing cell synthesizes most of the available soluble nutrient materials into new cytoplasm (assumedly made of molecules wherein proteins and lipoids are intimately combined) and new mitochondria of similar composition (Guilliermond, Mangenot, and Plantefol (26)) as well as new nuclear material. Meristematic cells characteristically are polyhedral in form. Each contains a nucleus that is large in comparison to the size of the cell and is surrounded by the cytoplasm wherein mitochondria show as small rods (Fig. 1, A). Vacuoles are numerous (Went (48)), small, and often elongated. They contain a thick, homogeneous, stable solution that stains vitally as a whole with neutral red or various other basic dyes.

In buds of healthy plants, the cells enlarge rapidly as the vacuoles imbibe water, swell, and fuse. During that period of active growth, mitochondria retain their rod shape and they evidence no physiological activity beyond dividing. As growth slackens, cells differentiate from the anatomical, histological, and physiological points of view.

In vascular tissues, cells forming wood vessels or sieve tubes have been given more attention than the cells in the wood parenchyma or the companion cells in the phloem. These cells, which evidence their physiological activity through the perpetual and striking shifting of their vacuolar material in the cytoplasm and respond readily to pathological stimuli by forming phenolic compounds, deserve careful consideration. Unfortunately, studying the phloem in diseased plants is difficult, phloem elements being transitory (Dufrenoy (14)).

Green tissues are made of cells where a certain proportion of the mitochondria differentiate into plastids, which, by forming chlorophyll, become

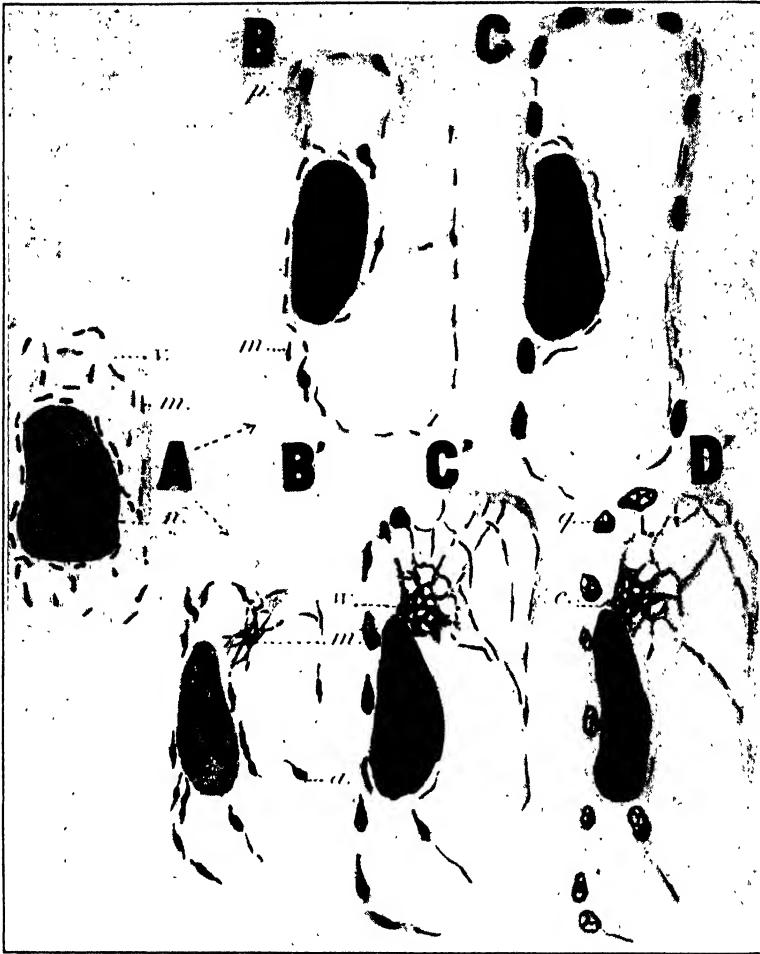


FIG. 1. Effect of virosis on the evolution of the embryonic cell. Starting from the meristematic condition represented in cell A, (with a large nucleus (n) some of the mitochondria (m) should normally differentiate into chloroplasts ((p) B), while the elongated small vacuoles (v) should coalesce and swell into a few large vacuoles (B and C). Under the influence of a virosis, starting from the same meristematic condition A, we see most mitochondria store in starch grains (a), thereby becoming amyloplasts (B') while some mitochondria (m) clump together along cytoplasmic strands separating a cluster of small vacuoles (w) (B' and C'). That vacuolated area of the cytoplasm, being highly refringent and staining most deeply, looks like an "inclusion" ((e) D'); plastids disintegrate ((q) D').

photosynthetically active (Fig. 1, B); they form starch when exposed to light in the presence of  $\text{CO}_2$ . Cells located deeper, which develop into storage, differentiate some of their mitochondria into amyloplasts, condensing translocated carbohydrates into starch grains.

## EFFECT OF VIROSES ON MITOCHONDRIA

What proportion of mitochondria of each cell differentiates into plastids depends on the metabolism. A cell does not manifest many forms of activity at the same time; dividing cells do not store starch; and, conversely, cells crowded with starch do not divide until they have translocated their starch out, in which case the amyloplasts revert to their original condition of mitochondria (Dufrenoy (13)).

The evolution of a great percentage of mitochondria into starch-storing amyloplasts is the first constant cytological response of the cell to growth inhibition in buds or in root tips of plants affected by virosis (Fig. 1, B) of any dwarfing disease (Dufrenoy (7)).

In meristematic tissues, starch storage is concomitant with decreased respiration. Buds of mosaic-affected tobacco use only 4 cubic millimeters oxygen per milligram dry weight per hour, whereas buds of healthy looking tobacco use nearly 6 (Dufrenoy (16)). Plastids may develop in excessive numbers in virus-affected tissues where normally few, if any, would develop. Esau (22) found more "sieve-tube plastids" in the beets affected by curly top than in normal beets. C. F. Lackey and we found that the virus of curly top induces early differentiation of mitochondria into amyloplasts, in post-meristematic cells, and on the contrary, inhibits the differentiation of the "starch sheath" around vascular tissues.

## HYPERTONIC DISEASES

Inefficient utilization of the products of photosynthetic activity may be responsible for both leptonecrosis and for the "high pressure developed by the abnormal accumulation of the sugars" (Carsner and Stahl (4)) in the tops of beets affected by curly top, in tomatoes affected by yellows, and in potatoes affected by leaf roll, where starch, photosynthesized in the chloroplasts, can no longer be translocated away, while many other unutilized products (phytosterol) are also stored in the vacuoles.

Vascular tissues may accumulate sugars, due to viroses, such as beet curly top (Carsner and Stahl (4)), or following the deficiency of some element such as boron (Haas and Klotz (28)) when starch translocation is inhibited.

*Mosaic.*—Early evolution of mitochondria into amyloplasts in virus-infected buds may leave few mitochondria available to develop into chloroplasts in leaf primordia. Many that begin to differentiate may fail to become photosynthetically active. Moreover, such chloroplasts as have differentiated may disintegrate. Starch grains in the plastids are the source of carbohydrate that the cells draw on. Starch usually is translocated out of the chloroplasts during the night, but early in the day the insolated chloroplasts of green cells are again replete with starch grains. In mosaic-

affected cells, even after a period of illumination, the starch grains are thin, elongated, and surrounded by some less condensed carbohydrate, which does not show the staining reactions of starch.

But, while the cell seems to draw freely on its reserves of starch and sugars, it can use up only a small fraction of its proteins. Aspects of plastids in virus-affected cells suggest local proteolytic disintegration: a crescent-shape part of the plastid, wherein traces of starch grains are preserved, with few small oil droplets in between, keeps its normal staining reactions (Fig. 1, D and Fig. 2, C) but the greater part of the plastid is swollen into a

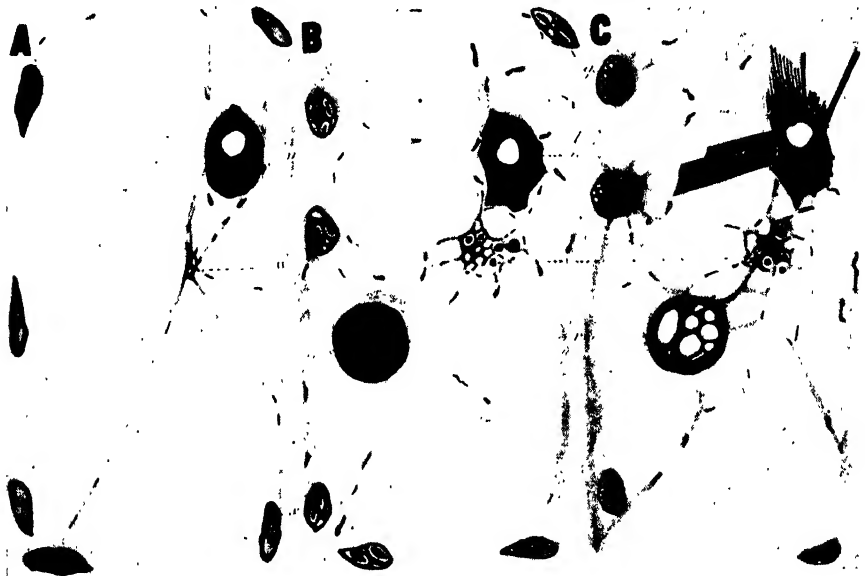


FIG. 2. Effect of a virosis on a palisade cell of *Nicotiana tabacum*. A. Formation of small vacuoles (w); the cytoplasm around becomes highly refringent, the rest of the cell being apparently unaffected and the plastids containing starch grains (s). B. The refringent area of the cytoplasm forms a network of strands, (along which mitochondria are actively dividing) around the small vacuoles (v) within which the vacuolar solution is easily flocculated into precipitates. Other large vacuoles may show vacuolar inclusions (r). Starch (s) is being translocated out of plastids, which assume a vesiculated appearance. C. Amino acids (mostly tyrosine (t) and leucine (l)) crystallize within some part of the cytoplasm. Plastids (q), almost entirely depleted of starch, undergo a partial disintegration and swell. The nucleus (n) becomes distorted.

dimly outlined mass, staining chiefly with erythrosin. This swelling of plastids can easily be watched by mounting in the Ringer solution some pieces of epidermis stripped off the margin of discolored area of iris leaves or of gladiolus flowers. Plastids swell in virus-infected cells of discolored areas much quicker than in neighboring cells from less altered areas, due probably

to a greater abundance of free hygrophilous lipoids. Swollen plastids generally show a green, crescent-shape portion, wherein small oil droplets show brightly, and a diffuse swollen gel mass; brownian movements have been described (Beauverie (1); Dufrenoy (8 and 9)).

#### ACCELERATED RESPIRATION OF GREEN CELLS AFFECTED BY MOSAIC

Mosaic diseases so impair photosynthesis that cells develop a hypotonic condition as the vacuolar solution is depleted of sugars. Cells depend for their metabolism on the splitting of previously photosynthesized materials or on the translocation of water-soluble material from neighboring cells. In these, as in the starved cells studied by Reed (40), "the kinetic energy necessary to carry this process must be generated by respiration."

Using Warburg apparatus, we found, with Genevois (25), that light green areas of mosaic leaves use more than 4 cubic millimeters of oxygen per mg. of dry weight per hour, while dark green areas of the same leaves use less than 4 and green tissues of healthy leaves use about 3. A respiratory activity as high as  $I_{O_2} = 4$  was found in leaves that had not yet developed external symptoms of mosaic, but in which the cells already showed the fine vacuolization characteristic of mosaic.

The higher respiratory intensity in mosaic cells is associated with a distribution of the vacuolar material between numerous small vacuoles, as contrasted with its presence in one large central vacuole in the healthy cell. Numerous narrow vacuoles show in the cytoplasm of epidermal cells of *Nicotiana glutinosa* 24 hours after the virus from *N. tabacum* has been rubbed on the leaf. In that exceptional case, general vacuolization is soon followed by the collapse of the cells. Necrotic lesions appear where the virus has penetrated, and check its generalization. Conversely, viruses that become systemic kill few, if any, cells. Most infected cells survive long enough to respond first by localized vacuolization and next by further localized biochemical changes, causing the formation of the cell inclusions.

#### THE CYTOPLASM VACUOLE INTERFACE

The living processes may be considered to take place mainly along the surfaces of contact between cytoplasm and vacuoles; local alteration of the cytoplasm, featuring "inclusions," may result from the breaking down of the adjustment of the two phases in contact along the interphase. (Fig. 1, B' to D'). Much may therefore be grasped from the study of contact surfaces between cytoplasm and vacuolar material in living cells from leaf epidermis of tobacco, iris, or hemerocallis, or flowers of gladiolus, which are transparent enough for their contents to be studied under the highest magnification of the microscope or of the ultramicroscope (Dufrenoy (17)). Neither the cytoplasm itself nor the vacuolar contents of a living cell are

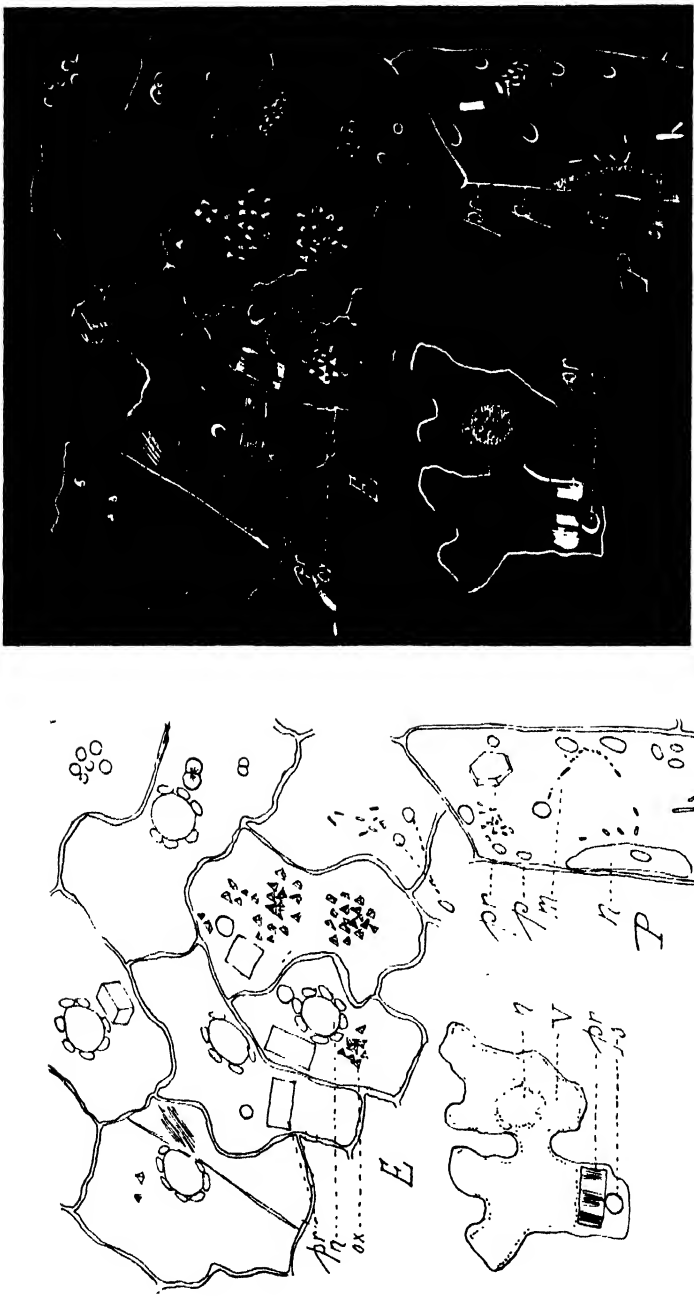


FIG. 3. Microscopic and ultramicroscopic aspects of (E) epidermal, and (P) palisade cells of mosaic leaf of *Nicotiana tabacum*. (n) nucleus; (p) plastids; (pr) tyrosin crystals (striated bodies); (o) vacuolar inclusions (possibly mixtures of lecithin and sterols; (ox) calcium oxalate crystals within the vacuoles (v).

visible under the ultramicroscope. The flowing of the cytoplasm between vacuoles is made evident through the bright oil globules that it carries along, but the cytoplasmic matter itself is optically empty; therefore, only the contour of mitochondria or plastids is illuminated out of the dark field. The

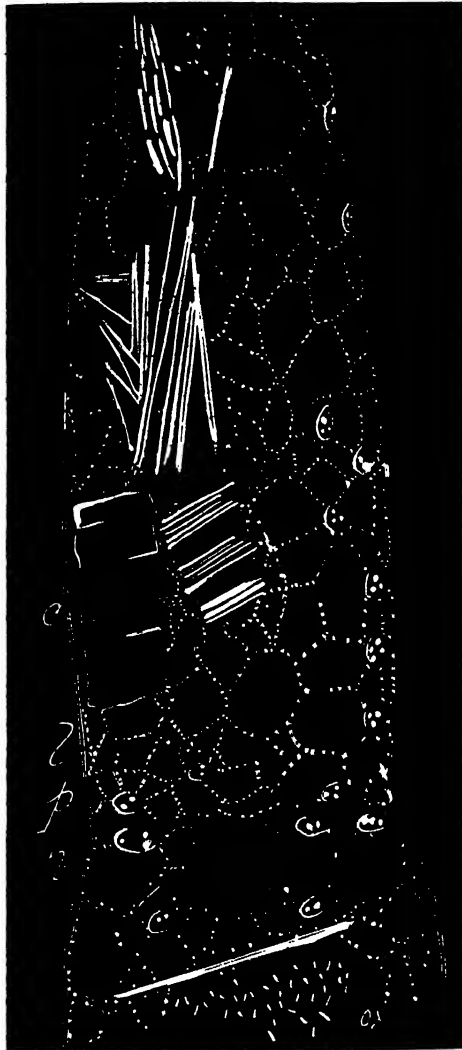


FIG. 4. Living hair cell from chlorotic area of mosaic leaf of *Nicotiana tabacum*, as seen under dark-field illumination. (n). Nucleus showing dimly. The separation between some cytoplasmic strands and vacuoles shows brightly. Normal cytoplasm itself is dark, but the included plastids (p) show from their bright contours. Fat globules (l) are brightly illuminated. Where proteolytic processes are active, dark cytoplasm is being gradually altered into bright proteic crystals (c).  $\times 2000$ .



FIG. 5. Cells of hair of mosaic tobacco leaf seen under the ultramicroscope.



FIG. 6. Beginning of the formation of a vacuolated (*c*) inclusion being shifted along the cytoplasmic film between vacuoles (*v*) in a cell of a leaf hair of tobacco affected by mosaic; (*n*) is the nucleus.



vacuolar solution also is optically empty but for crystals and, eventually, some vacuolar inclusions that may float in it.

Figures 3, 4, and 5 show the sandy crystals of calcium oxalate brightly illuminated within the dark vacuole solution. The interphase between the cytoplasm and the vacuolar material usually does not show on the dark field, so that we know of the cytoplasmic film only from the flow of the bright oil droplets carried along cytoplasmic currents. However, the modifications

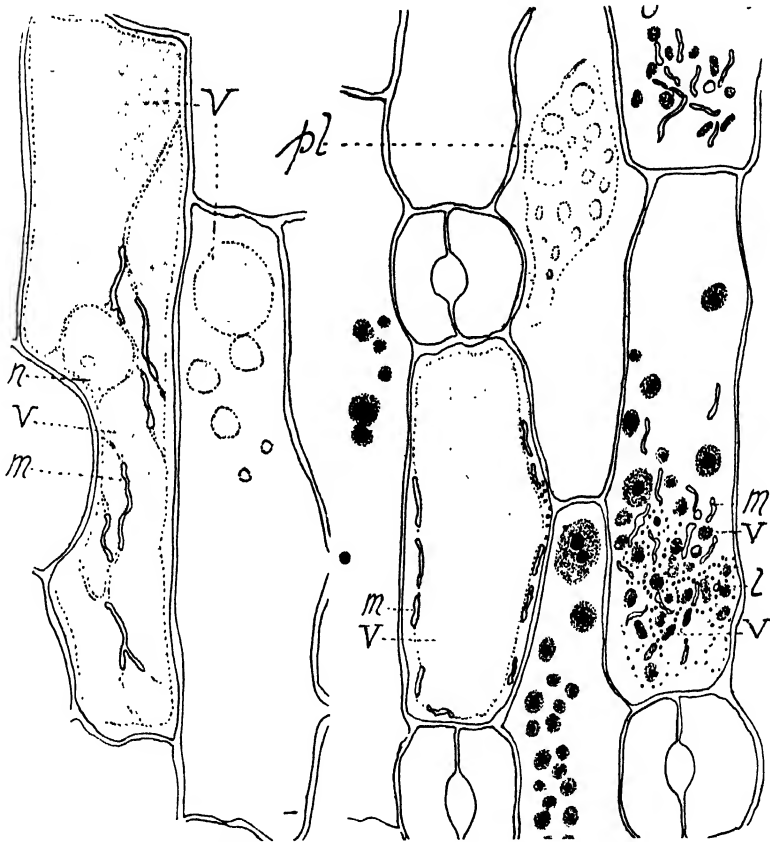


FIG. 7. Piece of living epidermis stripped from chlorotic area of leaves of *Hemerocallis* sp. stained in isotonic neutral-red solution. Normal cells show one or a few large vacuoles (*v*) surrounded by cytoplasmic strands where mitochondria (*m*) show as bright threads. Affected cells show a cluster of small vacuoles (*v*) within a network of cytoplasmic strands where short mitochondria are densely interwoven and refractive fat globules (*l*) are numerous. The vacuolar solution of large vacuoles stains pink, as a whole, with neutral red and is shown by stippling; that of the small vacuoles is partially precipitated into deeply staining intravacuolar spherical bodies (shown black). Necrotic vacuolated cytoplasmic bodies (*pl*), where the vacuolar contents can no longer be stained by vital dyes, are seen in some cells.  $\times 800$ .

brought about by the virus may result in the cytoplasm vacuoles' interphase becoming bright. This shows in the leaf hair of *Nicotianum tabacum* (Fig. 4) where a bright interface outlines the small clustered vacuoles. The cytoplasm, normally optically empty, gradually becomes bright where it is altered into bright crystals of needle-like tyrosin or plate-like leucine. Tyrosin crystals show also as striated bodies in glandular cells at the tip of a leaf hair (Fig. 5). (Dufrenoy *et al.* 19 and 20.)

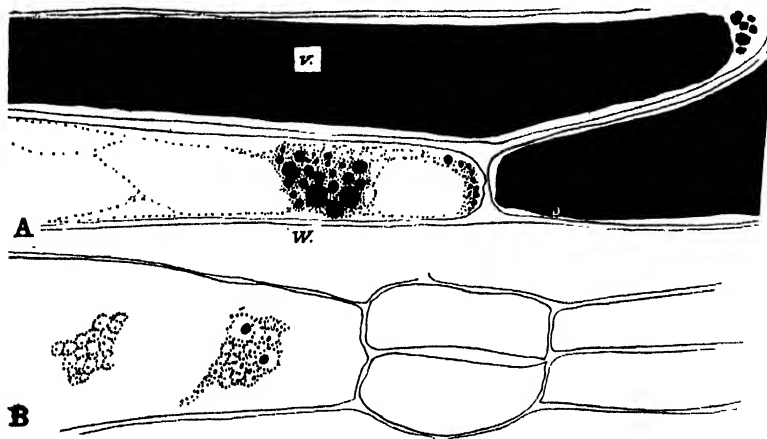


FIG. 8. A. Piece of epidermis of mosaic leaf of *Iris tectorum*. Vital staining with neutral red demonstrates the large central vacuole (*v*) in cells covering green areas and the clustered small vacuoles (*w*) in a cell at the margin of a discolored fleck (vacuolar content shown black). The vacuoles (*w*) are outlined by the numerous oil droplets present in the cytoplasmic films between the small vacuoles.  $\times 800$ . B. Epidermal cell of badly discolored fleck on *Iris germanica*; the contour of the small clustered vacuoles is outlined by the rows of oil droplets in cytoplasmic strands. The vacuolar solution does not stain as such with neutral red, but is precipitated as vacuolar precipitates (shown black).  $\times 800$ .

#### LOCAL MODIFICATION IN THE CYTOPLASM

The gradual building up of a vacuolated "inclusion" through the clumping together of mitochondria in between clustered vacuoles is best observed in hairs of tobacco (Fig. 6) or in the large epidermal cells of iris or hemerocallis leaves or in those of gladiolus flowers. Pieces of epidermis stripped off the margin of a discolored fleck on mosaic hemerocallis (Fig. 7) or iris leaf (Fig. 8 and 9) show that the epidermal cells covering the green area are mainly occupied by one or a few large vacuoles surrounded by a cytoplasmic film wherein long slender mitochondria are evenly distributed. Cells from discolored areas show a cluster of small vacuoles within a network of refractive cytoplasmic strands wherein oil droplets are numerous, and short mitochondria are densely interwoven. The same patterns,

as are easily made out in the living cell, can be stained after killing with mitochondria technic, as shown in figures 9-12. The refractive indices of



FIG. 9. Left: Epidermal cell of *Iris tingitana* showing an early stage of vacuolated-body formation, where short mitochondria cluster along a network of cytoplasmic trabeculae (c), with numerous small vacuoles in between. The nucleus shows at n. Right: Part of a cell where mitochondria are evenly distributed; many of these differentiate into plastids (p).  $\times 1500$ .

the "vacuolated cytoplasm" and its crowding with oil droplets (Dufrenoy (8), Clinch (5)) suggest a disintegration of the lipoproteins; a weak solution of eosin in Ringer fluid stains the "vacuolated cytoplasm" and the nucleus red, while the bulk of the cytoplasmic films remain unstained. The stained part of the cytoplasm may be spoken of as dead in contrast to the rest of the cytoplasm, which is still alive. That death may affect part of the cytoplasm in a cell, while the rest remains living, was recorded by Went as early as 1888. Local death in the cell can be observed in tissues starving

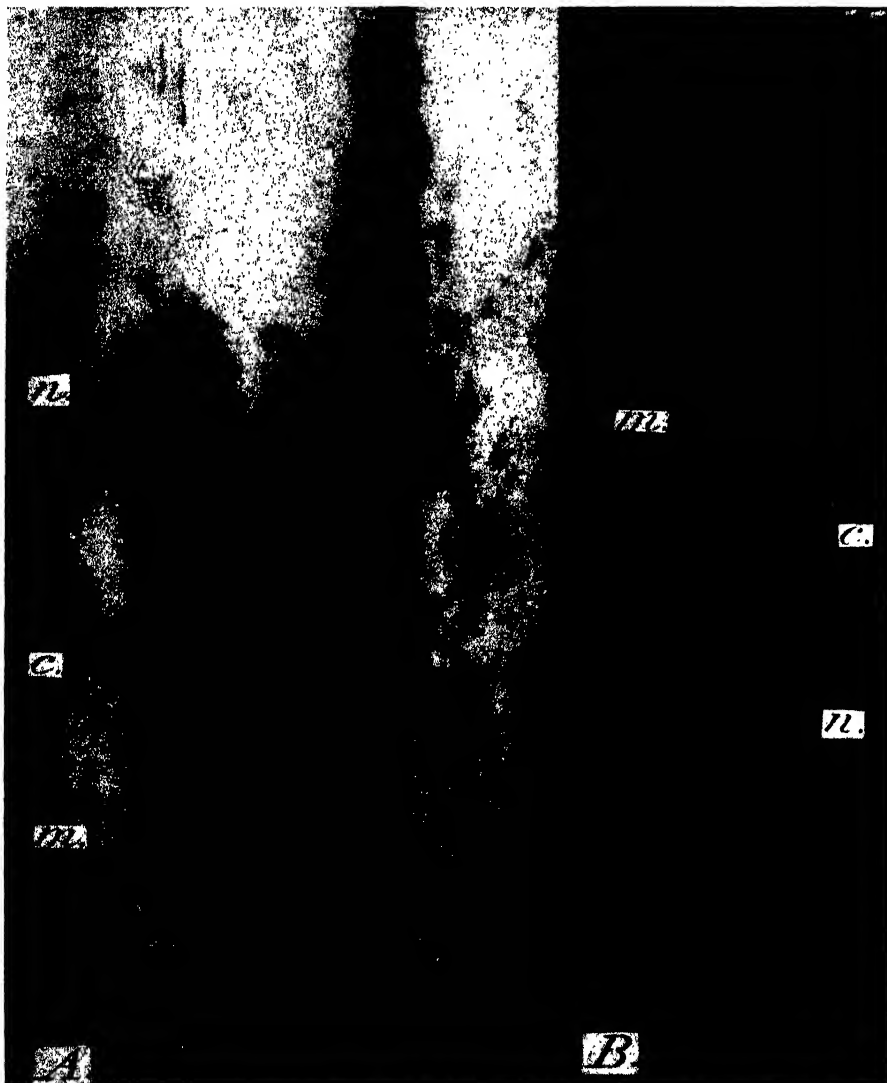


FIG. 10. *A*. Part of two epidermal cells of *Iris tingitana*. Left: Below the nucleus (*n*) a network of cytoplasmic strands stain deeply enough to obscure the mitochondria (*m*) inside; short mitochondria (*m*) can be made out on the border of that deeply staining cytoplasmic area (*c*). Long mitochondria show in the remaining part of the cytoplasm. Right: A cell shows a number of small clusters of short mitochondria which are so many foci for vacuolated-body formation. *B*. Mitochondria (*m*) are densely interwoven along the network of deeply staining cytoplasmic strands (*c*) around the nucleus (*n*).  $\times 1500$ .

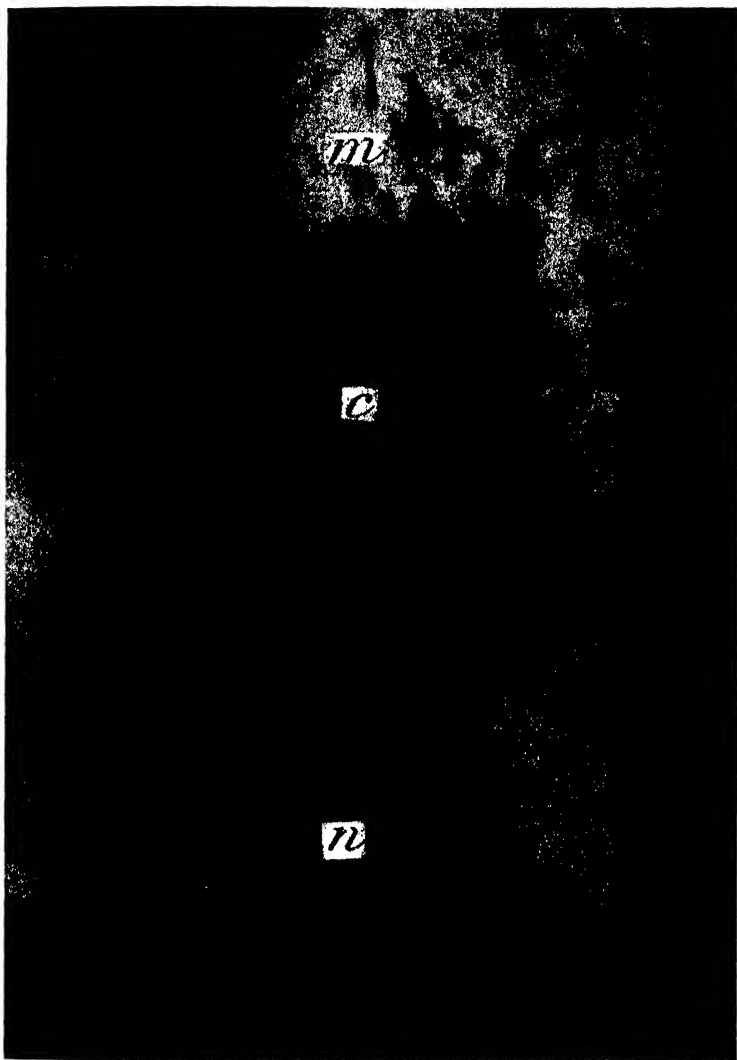


FIG. 11. Above the nucleus (*n*) of an epidermal cell of *Iris tingitana*, a certain cytoplasmic area (*c*) stains so deeply as to show as a vacuolated body with surrounding cytoplasmic strands, where mitochondria are fairly numerous (*m*).  $\times 3000$ .

under aseptic conditions or grown in the absence of some essential element (Dufrenoy (15, 18)). Sheffield (45) obtained cell inclusions by feeding molybdenum to the tissues. The similarity of localized cytoplasmic response to so many apparently different stimuli suggests a common basic cause, which we assume to be enhanced proteolytic activity. It was recently reported

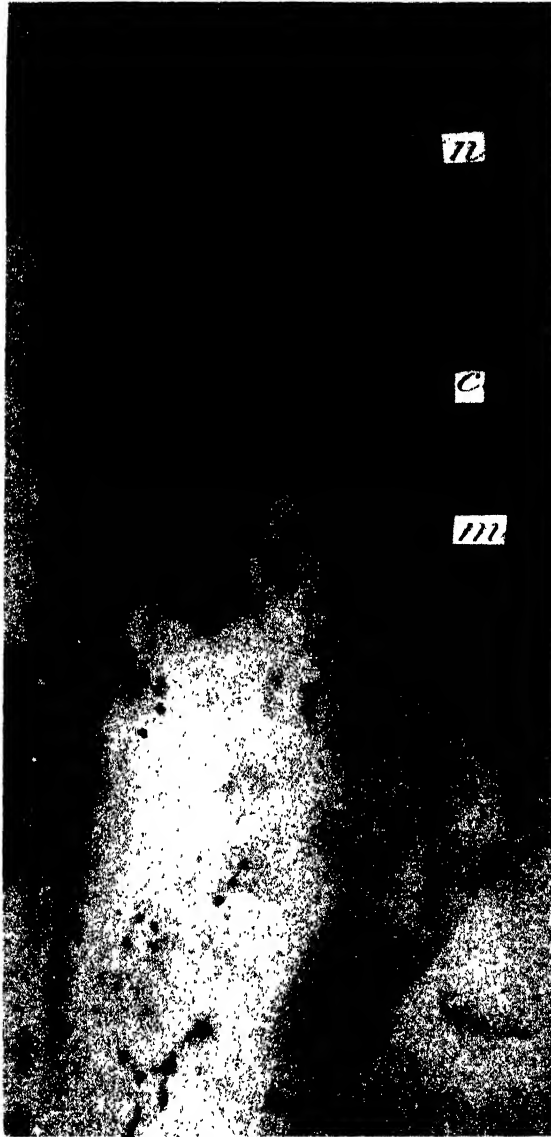


FIG. 12. Part of epidermal cell of *Iris tingitana* showing the ultimate stage of vacuolated body (c); deeply staining cytoplasmic area below the nucleus (n) is sharply made out from the remaining cytoplasm, where mitochondria (m) have assumed a bead-like appearance through active division.  $\times 1500$ .

that urea may accumulate in one of the vacuoles of a cell, there to induce active proteolysis of the immediately surrounding cytoplasm.

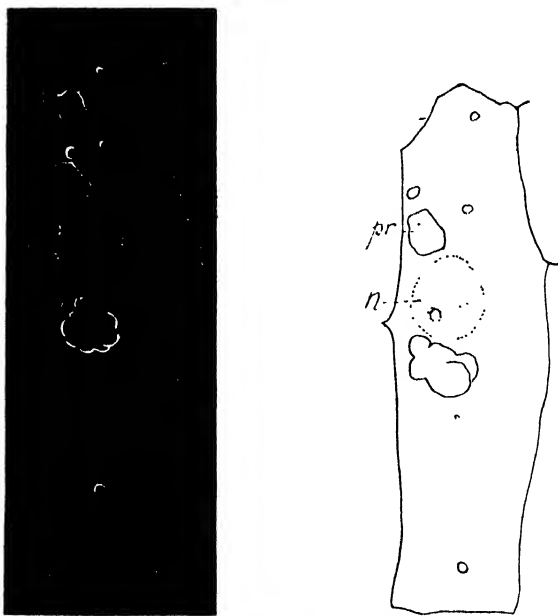
## PHYSICAL CONDITION OF THE VACUOLAR SAP

*a, pH.*—Viruses seem to shift the pH of the vacuolar solution from the more acid towards the less acid or the neutral. Calcium oxalate crystallizes as monoclinic crystals in the vacuoles of healthy *Nicotiana tabacum* or *N. glauca*, where the pH may be 3 or less. It crystallizes as tetrahedrons in mosaic-affected cells, suggesting a pH higher than 5.

Similar evidence is obtained from the purple or blue flecking of red or pink gladioli by mosaic and, better still, by the "breaking." Petals of pink gladioli owe their color to the anthocyanol in solution in the large central vacuole of each epidermal cell. Mosaic causes anthocyanol to crystallize out of its vacuolar solution into a purple spherocrystal, giving the affected epidermis a slaty appearance. Experimental crystallization of that anthocyanol may be obtained by buffering the solution of pink cells to pH 8.

The lowering of acidity by the virus may be correlated to increased rate of respiration, oxidizing further organic acids to CO<sub>2</sub>.

*b, Stability of the Vacuolar Solution.*—In the large vacuoles of epidermal cells of iris or hemerocallis leaves affected by mosaic, the vacuolar solution stains readily as such by neutral red; but it is precipitated as vacuolar precipitates within the small vacuoles that are clustered in the "vacuolated cytoplasm."



FIGS. 13-14. Cell of hair of mosaic leaf of *N. tabacum* showing, under the microscope (right) and the ultramicroscope (left): The nucleus (*n*), a lobed inclusion, and crystals of leucine (*pr*).

*c, Specialization of the Vacuoles.*—"When a cell contains several vacuoles these may differ as to their contents" (Went (48)). This is especially true of cells affected by virus or other causes of trouble. Vital staining with neutral red or Nile blue, as well as microchemical tests, confirm the coexistence, within the same cell, of vacuoles differing as to the pH and contents of the solution within. Accumulation of phenolic compounds in small clustered vacuoles occurred in epidermis of *Nicotiana tabacum* where we inoculated the "yellow mosaic" virus (Dufrenoy (16)). Ordinary green mosaic induced slower but more complicated changes involving calcium oxalate and presumably phytosterol (Figs. 13 and 14) with other sterids, lecithins, and phosphatids. Cells of *N. tabacum* may contain spherical or vesiculated inclusions (presumably made of mixtures of lecithin and sterols or possibly involving a colloidal form of carbohydrate not only as a result of mosaic diseases, but also when abnormal chromosomic constitution inhibits normal growth or differentiation. These inclusions are to be compared with those described by Reed and Dufrenoy (41) in mottle leaf of Citrus. Such bodies, which have been extensively studied by Guilhermond (27) seem to be common. (Fig. 15, *r*.)

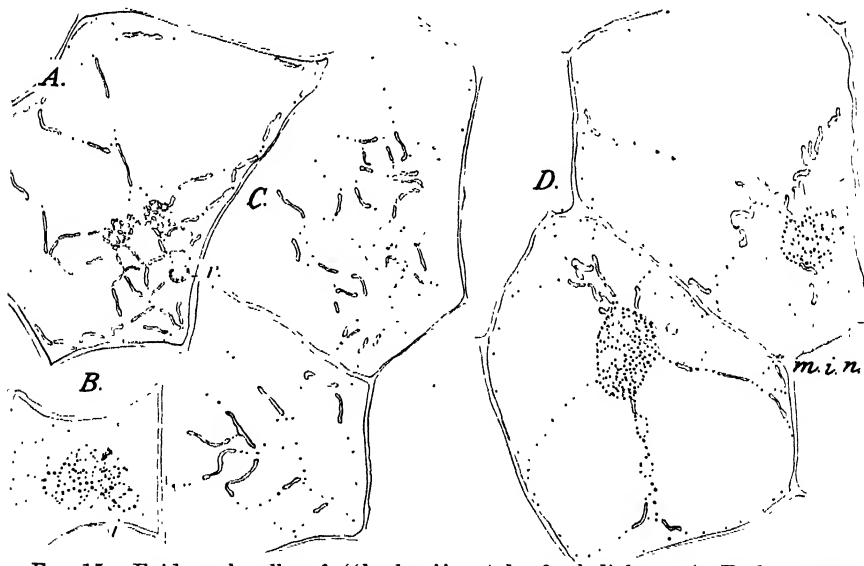


FIG. 15. Epidermal cells of "broken" petal of gladiolus. *A*. Early stage of vacuolization of the cytoplasm, showing the crowding of short mitochondria and oil droplets, and formation of vacuolar inclusion (*r*). *B* demonstrates the oil droplets (*i*), after staining with indophenol blue. *C* and *D* show a later stage of the vacuolization and accumulation of oil droplets (*i*) near the nucleus (*n*). Mitochondria (*m*) are short near the vacuolated cytoplasm, but they retain their normal length on the slender cytoplasmic films separating the large vacuoles. *C* was observed by focusing on the lower level, *D* on the upper level of the same two living cells.  $\times 1500$ .



## THE INTRACELLULAR VIRUS

Squeezing out the contents of animal cells showing inclusions as the result of virus infection made it possible for Borrel (3) and Ledingham (35) to demonstrate, by proper staining, minute granules  $0.2\mu$  in diameter that may represent the virus. Squeezing the cytoplasm from cells under the Meves killing fluid, mordanting with iron tannate and staining with carbolfuchsin, following the Borrel technic, enabled us (17) to obtain photomicrograph (Fig. 16) of similar granules from mosaic cells of tobacco leaves or of

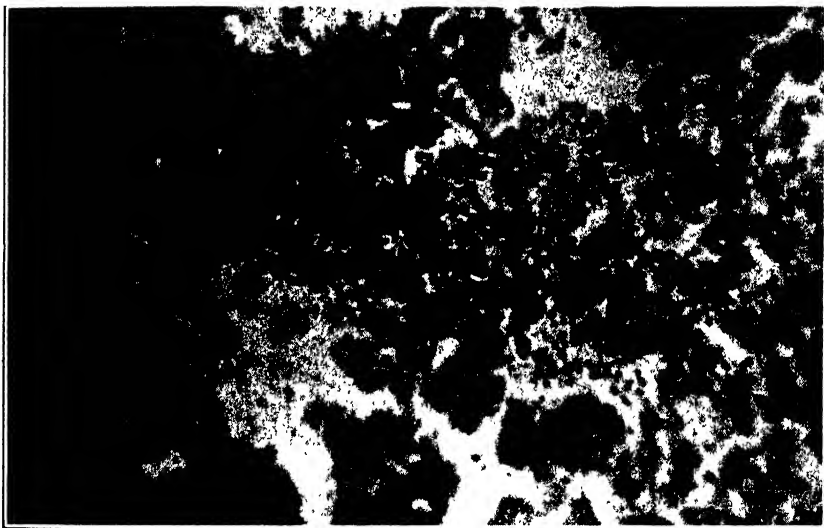


FIG. 16. Photomicrograph of the cytoplasmic film squeezed out of a mosaic epidermal cell of tobacco fixed with the Némec killing fluid, mordanted in iron tannate, and stained with Ziehl's fuchsin. The deeply staining granules showing on the hazy cytoplasmic background may be assumed to represent virus particles, considerably enlarged by a coating of adsorbed iron tannate.  $\times 2000$ .

"broken" tulip petals. Bewley (2), using a different technic, also sees the virus of tomato mosaic in the form of a granular deposit "with deeply staining fragments." B. M. Duggar (21) recently localized "the main virus concentration" "in the protoplasm" [of tobacco cells] "and not in the cell sap."

## RELATION BETWEEN CYTOLOGICAL RESPONSE AND RESISTANCE

When virus infection becomes systemic the response of the cytoplasm in each cell tends to be localized to a small area, honeycombed by the formation of small, clustered vacuoles wherein phenolic compounds may accumulate. That vacuolated part of the cytoplasm may to all appearances die, but the

bulk of the cytoplasm in the cell continues to live, although its metabolism may be altered.

Such a situation is strikingly similar to that resulting from the infection of cells by haustoria of such parasites as develop a systemic infection, without causing necrosis. The cytoplasm around the haustorium may become finely vacuolated—it may be altered to the extent of being, to all appearances, dead; but the greater part of the cytoplasm in the infected cell remains alive, although it may manifest an altered metabolism.

Conversely, when a fungus infection fails to become systemic, but is responsible for such necrotic responses as are associated with resistance, cells in the vicinity of the hyphae collapse before they are ever penetrated by a haustorium, and neighboring cells accumulate phenolic compounds in a large central vacuole. Similar phenomena are observed whenever a virus fails to develop systemic infection, the best example being the early formation of phenolic vacuolar material in cells of *Nicotiana glutinosa* where local



FIG. 17. *S.* Longitudinal section through sun blotch lesion of cortex parenchyma of avocado, showing cells with brown phenolic vacuolar contents (*t*) between living cells, some of which are crowded with starch grains (*s*), while others are occupied mainly by a large vacuole (*v*), the contents of which stain as a whole with neutral red. (Professor Horne's material.) *P.* Transverse section through Citrus leaf showing a lesion of psorosis (Dr. Fawcett's material)—long, slender cells, surrounded by swollen pectic walls, and containing brown phenolic vacuolar precipitates (*t*) are crushed between mesophyll cells, showing plastids (*p*) and mitochondria (*m*).

nerosis develops as the result of infection by the virus of *N. tabacum* mosaic as studied on plants kindly made available by Drs. Takahashi and Shapovalov.

The accumulation of phenolic compounds in a circular layer of cells bordering each lesion is responsible for the "ring-spots" pattern of certain viroses (Dufrenoy (10)). For the definition of ring spots, see K. Smith (47).

The typical "sun-blotch" lesions of avocado (Horne (32)) are characterized by longitudinal series of cortical cells developing large vacuoles full of phenolic compounds; and similar "phenolic" cells are the most conspicuous features of the scaly bark disease of Citrus (Fig. 17), newly recognized as due to a virus (Fawcett (23)).

#### DISCUSSION

The cytoplasm in the normal living cell looks so homogeneous that neither the microscope nor even the ultramicroscope can reveal anything of its intimate structure. In the homogeneous cytoplasm, mitochondria and eventually plastids (which proceed from mitochondria) show without any staining; vacuoles can easily be demonstrated through the use of vital dyes. We can therefore easily learn through the study of the architecture of the normal cell that a certain distribution and arrangement of mitochondria and vacuoles within the cytoplasm is linked up with the maintenance of life. We can even correlate certain readjustments to certain modifications of the cell metabolism, but we can learn little of the structure of the cytoplasm itself, of mitochondria, or of plastids, through the direct observation of the healthy cell.

Agents that result in the partial breakdown of that homogeneous structure or that interfere with the making up of the cell constituents may reveal some clue as to what materials comprise the cytoplasm and plastids. Viruses are most effective in that respect. Through the study of the virus-infected cell we should learn much about the structure of the healthy cell.

Examination of a great number of living cells from different tissues—some healthy, others diseased—makes it evident that the more active the life processes in the cell (1) the greater the area of interfacial contact between cytoplasm and vacuoles; and (2) the more rapid the changes of relative positions of cytoplasm and vacuoles in the cell.

Be it physiological, as evidenced in glandular cells of the hairs of carnivorous plants (*Drosera*), or pathological, as in cells affected by microscopic parasites or by viruses, enhanced biochemical activity is evidenced by the division of the bulk of involved cytoplasm into a number of slender films, spreading and forking into the vacuolar material, as so many partitions, which divide a large vacuole into a number of smaller ones. When the

partitioning off is carried to the extreme, the cytoplasm assumes a honey-combed appearance, which has been described in virus-affected cells under various names, but corresponds to the similar structures resulting from local proteolytic activity induced by the penetration of fungus haustoria, or that can even be observed in cells of tissues starving under aseptic conditions.

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# THE MODE OF INFECTION OF DIPLOCARPON EARLIANA AND MYCOSPHAERELLA FRAGARIAE

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## INTRODUCTION

During the course of an investigation of the influence of seasonal variations on the amount of infection with the strawberry leaf spot (*Mycosphaerella fragariae* (Tul.) Lindau) and scorch (*Diplocarpon earliana* (Ell. & Ev.) Wolf) under Louisiana conditions, an inconsistency in the results obtained from inoculation experiments was noted that could not be accounted for on the basis of environmental factors. It was discovered that this inconsistency was due to the methods used in inoculating the plants. It was noted that when the inoculum was sprayed on the plant by means of an atomizer (in which case spores would lodge both on the upper and lower surfaces of the leaves) good infection was obtained, whereas when the inoculum was placed only on the upper leaf surface, there was either no infection at all, or only a very slight amount of it. This led to a rather detailed investigation of the mode of infection of these two serious parasites of the strawberry, the methods and results of which form the substance of this paper.

## HISTORICAL

The literature bearing directly on this subject is rather limited. Most of the papers dealing with the strawberry leaf blights either make no mention of the mode of infection, or simply state that the spores fall on the leaves, germinate, and enter the leaf tissue, without giving any definite information as to whether infection takes place through the upper or the under leaf surfaces and whether the ingress is through the stomata or by penetration of the epidermis. Garman (4) states: "As these latter (spores) ripen, they are set free, are scattered upon fresh leaves by winds and rains, to germinate there, push into the leaves, and form new spots." The use of the term "push" would perhaps suggest that Garman considered ingress to be by penetration of the epidermis rather than stomatal. Two papers, Dudley's (3) on leaf spot, and Wolfe's (8) on scorch, have a direct bearing on the mode of infection, and these are reviewed somewhat in length as the findings of these workers are, for the most part, at variance with those of the writer.

Dudley (3) claims infection with *Mycosphaerella* may take place through both the upper and the lower leaf surfaces and that the germ tube enters not through the stomata but by *boring* through the epidermis. He

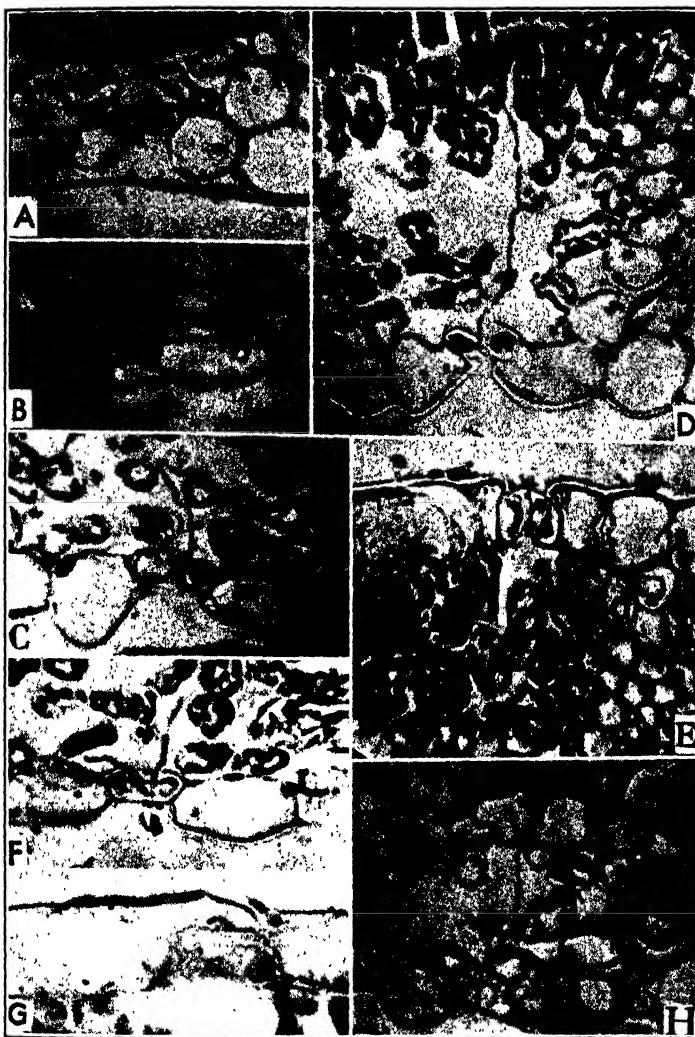


FIG. 1. Mode of infection in *Mycosphaerella fragariae*. A and B. Very early stages of infection. Young germ tubes just entering stomata of the lower epidermis. Three days after inoculation.  $\times 386$ . C. Germ tube has entered through a stoma and is curving around a mesophyll cell. Five days after inoculation.  $\times 500$ . D. Germ tube has entered through stoma of under epidermis and is growing upward toward the palisade cells and upper epidermis. Four days after inoculation.  $\times 386$ . E. Typical stoma on the upper epidermis of the Klondike variety of strawberry.  $\times 386$ . F and H. Lower epidermal stomata with 2 germ tubes (or branches of the same germ tube ?) entering. Seven days after inoculation.  $\times 500$ . G. Germinating spore on the upper epidermis, its germ tube curving toward an upper epidermal stoma.  $\times 500$ .

states (p. 175): "Numerous infections were made in April and May, by placing both germinated and ungerminated conidia on the upper and the under surfaces of young strawberry leaves, which we believed to be previously uncontaminated by the fungus. It was found that the germ tubes bored their way between the epidermal cells of the upper surface, but they were not observed to enter by the stomates of the under surface, although infection took place readily from that surface. Entrance by the stomates was certainly not the usual mode of attack." No photographs or other illustrations of germ-tube penetration are shown, and the variety or varieties experimented with is not designated. This latter point is important, for in the present investigations it was found that the leaves of some strawberry varieties have stomata on both surfaces.

The writer's findings are not in accord with those of Dudley. Germ-tube ingress was repeatedly found to be stomatal (Fig. 3); if ingress can occur also by penetration of the epidermis, it was never observed during the course of these studies.

Wolf (8), in describing the development of acervuli and conidia of both the strawberry fungus, *Diplocarpon earliana*, and the rose fungus *D. rosae*, states (p. 10): "The essential features in the development of the conidial stage of the strawberry fungus and the rose fungus are entirely similar. In the case of each, infection occurs by direct penetration of the germ tubes from conidia which lodge and germinate on the upper leaf surface. Within a few days this hypha will have developed a subcuticular mycelium. Meanwhile certain branches will have extended downward between the epidermal cells and between the subjacent palisade parenchyma and mesophyll." Dodge (2) also reports the formation of a subcuticular layer of mycelium in the case of the rose fungus.

The writer did not include the rose fungus in his studies, but his findings on the mode of infection of the strawberry fungus do not agree throughout with those of Wolf. He agrees with Wolf that ingress occurs by direct penetration of the epidermis by the germ tubes, but finds that infection takes place primarily, if not wholly, through the lower, rather than the upper, leaf surface. Furthermore, no subcuticular mycelium was ever found to develop from the germ tube. The only subcuticular mycelium observed was the stroma from which the young acervulus arises and that makes its appearance at a later stage, just prior to the beginning of the young acervulus.

#### MATERIALS AND METHODS

The Klondike variety of strawberry was used in all the infection experiments with *Diplocarpon* and in all but two with *Mycosphaerella*. Potted plants were used in all cases. The inoculated plants were kept either in the laboratory by the window, in the greenhouse, or outdoors.



The inoculum consisted of conidial suspensions in water made from pure cultures of the respective organisms grown either on bean-pod agar in plates or on bean pods in tubes. The inoculum was smeared on the upper or lower leaf surface by means of a camel-hair brush. When the material was intended for histological studies, the inoculum was smeared on definite leaf areas marked with India ink. The inoculated plants were kept under moist bell jars for 2 to 3 days after inoculation.

The material for paraffin imbedding was fixed at different intervals from the 2nd to the 7th day after inoculation. Gilson's and the alcohol-acetic acid-formalin (100 cc. of 50 per cent alcohol, 6.5 cc. of formalin, and 2.5 cc. of glacial acetic acid) fixatives were used. The latter was found more satisfactory. Various stains were tried, including Pianeze III B (7), Dickson's (1), Ridgway's (5), Flemming's triple, Haidenhain's haematoxylin, and Stoughton's (6) thionin-orange G. Both the haematoxylin and the thionin stains proved satisfactory, but the latter was used most extensively because of the easiness and the shortness of the process.

#### EXPERIMENTAL

The experimental work was carried on simultaneously with both fungi but, for the sake of clearness, the results obtained with the two organisms are presented separately.

#### INFECTION EXPERIMENTS

1. *Diplocarpon earliana*.—The inoculation experiments with *D. earliana* and the results obtained are, for convenience, summarized in table 1. The lower-surface inoculations, in all except the first experiment, resulted in very heavy infections. Hundreds of spots developed in every case on every leaflet inoculated on the lower surface in 10 to 14 days. These spots would coalesce and "scorch" the leaves, often killing them completely in 18 to 21 days. Acervuli and conidia developed in abundance, and always on the upper surface. Dodge (2) found that the acervuli of *D. rosae* occasionally occur on the lower leaf surface, and he considers this as an indication that infection sometimes may occur naturally through that surface. In these experiments where infection was known to have taken place through the lower surface, the acervuli were found to develop only on the upper surface.

The relatively light infection in experiment I (Table 1) is thought to be due to the fact that the inoculated plants were not placed under moist bell jars, and there was not sufficient moisture for the spores to germinate.

The upper-surface inoculations resulted either in no infection at all (Experiments I, III, IV, V and VII, Table 1), or in very light infection (Experiments II and VI, Table 1). It is not certain whether the infection in

TABLE 1.—Results of inoculations with *Diplocarpon earliana* on the upper and under surfaces of the leaves

Experiment number	Date	Location	Number plants inoculated		Results	
			Upper	Under	Upper	Under
I <sup>a</sup> . . .	2/15/29	Greenhouse	3	3	No infection	4 spots on one plant, 10 on another, and no infection on third
II . . .	3/11/29	Greenhouse	3	3	Very light infection, only occasional spot here and there (Fig. 2, A and B)	Hundreds of spots on every inoculated leaf (Fig. 2, A and B)
III . . . . .	11/19/29	Laboratory	1	1	No infection	Hundreds of spots on every inoculated leaf
IV <sup>b</sup> . . . .	11/27/29	Greenhouse	2	2	No infection in 3 weeks	Minute spots appeared in 12 days. By end of 3 weeks, hundreds of coalescing spots "scorched" the leaflets
V . . . .	12/4/29	Laboratory	1	1	No infection	Hundreds of coalescing spots on every leaf
Check <sup>c</sup> . . .	12/4/29	Laboratory	1	1	No infection	No infection
VI . . . . .	1/9/30	Greenhouse	2	2	8 spots on one plant and 5 on the other	Hundreds of spots on every leaf
Check <sup>c</sup> . . .	1/9/30	Greenhouse	1	1	No infection	No infection
VII <sup>a</sup> . . . .	2/4/31	Laboratory	4 (18 leaflets)	4 (16 leaflets)	No infection	Hundreds of coalescing spots on each leaflet

<sup>a</sup> Inoculated plants not placed under bell jars.<sup>b</sup> Plants sprayed with sterile water and placed under bell jars 7 hours before inoculation.<sup>c</sup> Surfaces brushed with sterile water.<sup>a</sup> The same 4 plants were used for both upper and under surface inoculations, some of the leaflets on each plant being inoculated on the upper and some on the under surfaces and some being left uninoculated as checks.

these two experiments actually occurred through the upper surface, or whether it was due to accidental contamination of the lower surface. It may or may not be significant that when the inoculated plants were kept in the laboratory (Experiments III, V, and VII, Table 1) and extreme caution was used in the process of inoculations (all the overlapping leaflets were removed so there would be no touching between the upper and lower surfaces of the inoculated leaflets) no infection ever resulted from the upper-surface inoculations.

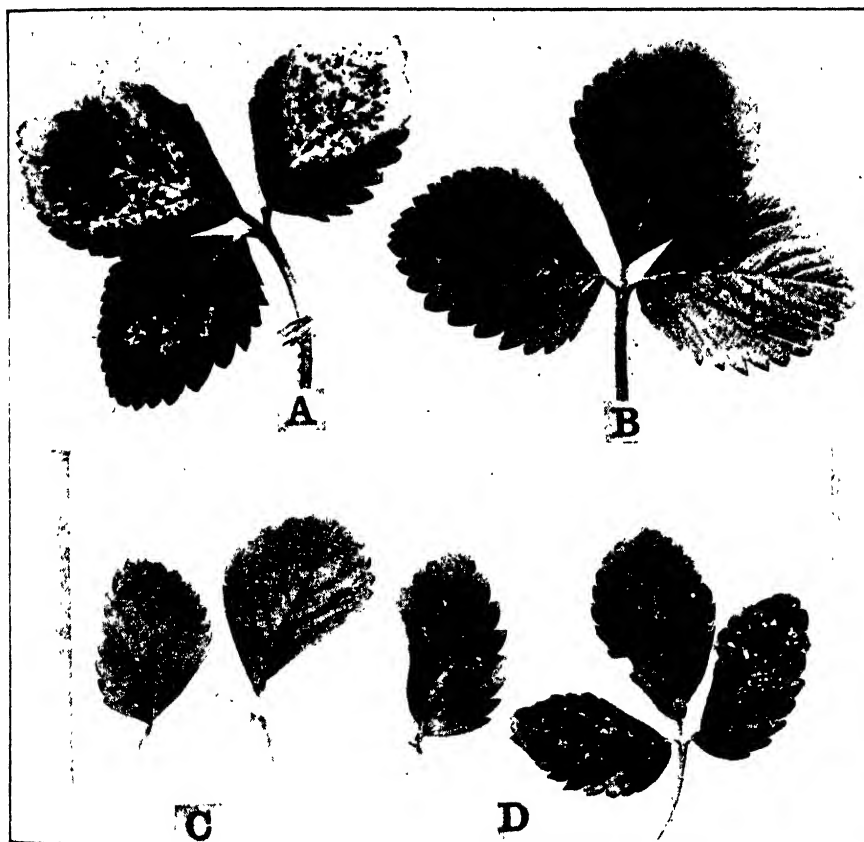


FIG. 2. A and B. Results of inoculating leaves with *Diplocarpon earliana*. A. Lower surface. B. Upper surface. C and D. Results of inoculating leaves with *Mycosphaerella fragariae*. C. Upper surface. D. Lower surface.

Since the inoculation was done by smearing the inoculum onto the surface by means of a camel-hair brush and consequently some rubbing of the surface and breaking of trichomes occurred, it was feared that some of the spots might be due to mechanical injury of the epidermis rather than

points of fungal infection. To test this possibility, check inoculations were included in some of the experiments (Experiments V and VI, Table 1) in which the brush was dipped in sterile water and rubbed on the leaf surfaces in the same manner as when inoculum was used. No spots developed and no injury to the surface, either to the upper or to the under, was noted in these cases.

#### HISTOLOGICAL STUDIES

Several methods were tried in the effort to determine the mode of infection. The Pianezze III B differential stain (7) was first tried. Pieces of the inoculated leaves were fixed in a mixture of equal parts of 95 per cent alcohol and acetic acid. After the chlorophyll was removed by several changes of 70 per cent alcohol, the bleached leaf pieces were stained, mounted, and examined. This method proved unsatisfactory, for the thick covering of trichomes on the under surface interfered with the field of vision.

Attempts were made to strip the epidermis of inoculated leaves and examine it to see how the germ tube enters. The strawberry leaf epidermis does not strip readily, however, and only very small pieces could be examined. Since the scorch fungus attacks, besides the leaf blades, also the petioles and the fruit-cluster stems, and since the epidermis of the latter 2 organs strips very readily, the method was modified and petioles and fruit stems were used. Beginning the second day after inoculation, pieces of the epidermis were stripped off, mounted in a 10 per cent solution of glycerine in S. T. 37 (Hexylresorcinol), stained on the slide with a very dilute solution of eosin, and examined. The fungus mycelium stains much more deeply than the epidermal cells and can be seen very easily. This method proved only partly successful. It did not show how the germ tubes enter, but it gave a strong indication that ingress was not stomatal. Hundreds of stomata were examined, but in no case was a germ tube found entering through a stoma. On the other hand, germ tubes often were observed growing over a stoma, or very near it, without entering.

The method finally adopted was to make paraffin sections of the inoculated leaves. While this method was long and time-consuming, as hundreds of slides were made and thousands of sections examined, the satisfactory results obtained justified its use. The material was killed and fixed at one-day intervals, beginning 24 hours after inoculation and continuing through the seventh day. Leaf material from natural scorch infection in the field also was included. This material represented the earliest visible stages of infection, very young spots, barely visible to the naked eye, and spots slightly older, of pin-point to pin-head size. The sections were cut from 6-12  $\mu$  thick and both parallel and vertical to the surface.

*Findings:* Over 2,000 sections of leaf material, fixed from the second to the seventh day after inoculation, were examined. In no case was the fungus found entering from the upper surface. It is not claimed that infection cannot take place through the upper surface; in fact, the results of some of the inoculations (Experiments II and VI, Table 1) might indicate that occasionally infection does occur through that surface. Under the conditions of these experiments, however, fungus ingress through the upper surface either did not occur, or was so rare that its occurrence was not observed in the examination of the sections. On the other hand, entrance through the lower surface frequently was observed (Fig. 3 and Fig. 4, C).

The germ tube is rather thick and of irregular diameter (Fig. 4, A and B) and stains somewhat lighter than the young branch hyphae arising from it after penetration. It enters the tissue by penetrating between the epidermal cells (Fig. 3 and Fig 4, C), and grows directly to the mesophyll

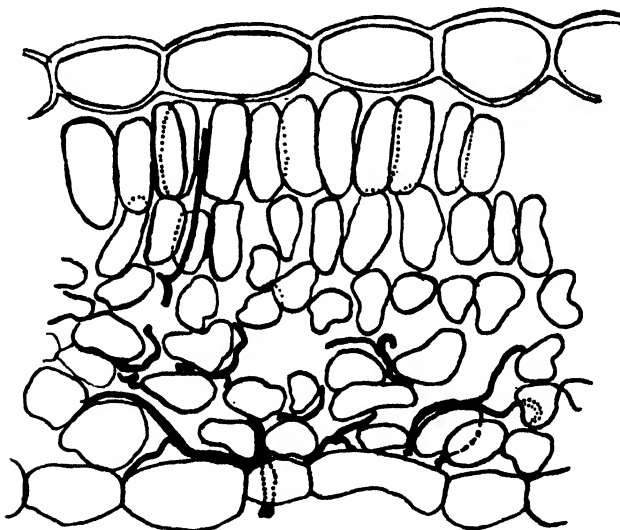


FIG. 3. Mode of infection with *Diplocarpon carliana*. Point of fungus ingress and relation of mycelium to host tissue are shown. Drawn from a photomicrograph made 5 days after inoculation.  $\times 386$ .

layer without forming any subcuticular layer of mycelium. No subcuticular mycelium was ever observed, either on the upper or the lower epidermis, either in sections of relatively young material (1 to 5 days after inoculation) or in sections of young spots (age of infection unknown) on naturally infected leaves collected in the field. It never was seen to enter by penetrating the epidermal cells, themselves, nor through the stomata. On the contrary, germinating spores often were observed in the immediate vicinity of stomata, sometimes lodged directly over them, the germ tubes

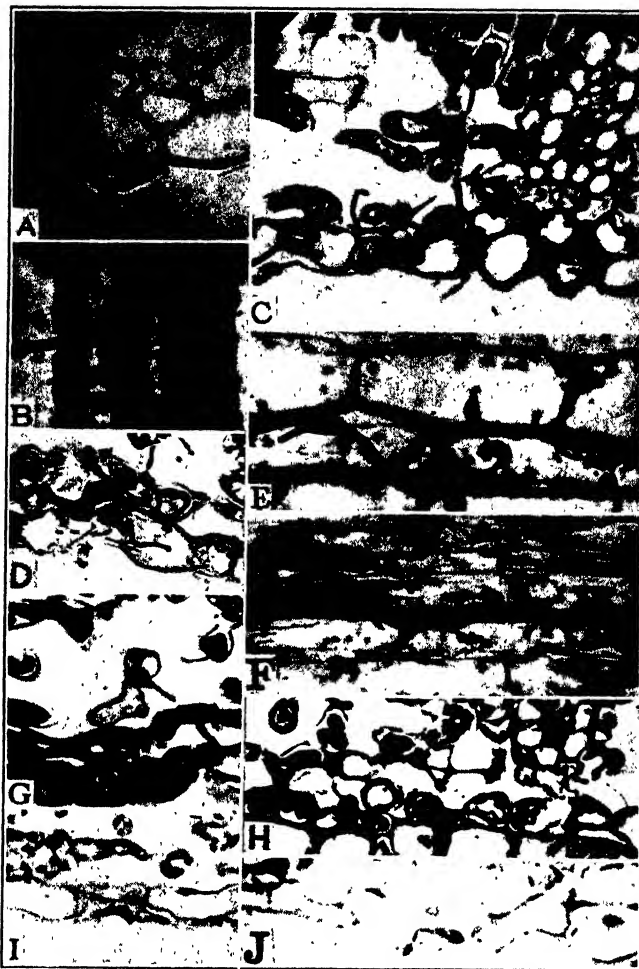


FIG. 4. Mode of infection in *Diplocarpon earliana*. A. Germ tube following the contour of a lower epidermal cell, its point pressing against the middle lamella. Material killed and fixed 3 days after inoculation.  $\times 400$ . B. Longitudinal section of fruit-cluster stem showing penetration by germ tube. Material killed and fixed 5 days after inoculation.  $\times 400$ . C. Cross section of leaf, showing mycelium in the tissue and point of entrance between 2 lower epidermal cells. The outside remnant of the germ tube is seen (slightly out of focus) extending from the point of entrance outward. Material killed and fixed 3 days after inoculation.  $\times 329$ . D. Haustorium (?) in epidermal cell.  $\times 320$ . E and F. Longitudinal sections of fruit-cluster stem showing haustoria in cells. Material killed and fixed 5 days after inoculation. E,  $\times 432$ ; F,  $\times 320$ . G. Club-shape hypha (haustorium mother cell?) pressing against a mesophyll cell that shows injury. H. Hypha similar to G, its thickened end pressing against a mesophyll cell and its pointed end inside another mesophyll cell. Both cells have collapsed. Material fixed 3 days after inoculation.  $\times 320$ . I and J. Germinating spores lodged over stomata, their germ tubes growing away from the stomatal openings.

growing away from, rather than toward, the stomatal opening (Fig. 4, I and J). Once inside the tissue, the germ tube branches rather freely, and gives rise to hyphae that grow between the mesophyll cells (Figs. 3 and 4, C). In the early stages, the mycelium is confined to the mesophyll layer. Later, branch hyphae grow upward toward the palisade cells and the upper epidermis (Figs. 3 and 4, C). In the beginning, the cells in the vicinity of the mycelium do not seem to be injured, judging from their appearance and staining reactions. When injury becomes apparent (collapsed or discolored cells), it is first manifested in the lower epidermal and mesophyll cells.

In the leaf tissue haustoria are not apparently abundant, at least in the early stages of infection. In all the leaf material examined, only 3 cases of structures resembling haustoria were observed. One is a hypha within an epidermal cell of the lower surface (Fig. 4, D). This may be considered as either an intracellular hypha or as a haustorium. Since the mycelium in the tissue was found to be intercellular, this structure is assumed to be a true haustorium, even though, in form, it is not typical of the definite haustoria found in the cells of the fruit stems (Fig. 4, E and F). The other 2 structures were observed in the mesophyll tissue of material killed 3 days after inoculation. One (Fig. 4, G) is a club-shape hypha with its thickened end pressing against a mesophyll cell. The cell is not collapsed, but apparently is injured, as shown by its staining reaction. This club-shape hypha is assumed to be a haustorium "mother cell." A similar hypha is seen in figure 4, H. Its thickened end is pressing against a mesophyll cell and its opposite end has penetrated a second cell. Both cells have collapsed and stain brown instead of deep purple, like the healthy cells.

In the tissue of the peduncles, on the other hand, haustoria were found in abundance (Fig. 4, E and F). These are very similar to those of *Diplocarpon rosae*, described by Dodge (2). They may be rather long and hypha-like, short and thickened, of irregular diameter, straight or curved, and with or without a sheath at the base (Fig. 4, E and F).

### Infection Experiments

II. *Mycosphaerella fragariae*.—As brought out in table 2, in which the results of the inoculation experiments with the leaf-spot fungus are summarized, infection with this fungus also occurs usually through the lower leaf surface. Either there was no infection (Experiments II, III, V, VI, VII, VIII, and X, Table 2), or very light infection, compared to that on the under surface (Experiments I, IV, IX, and XI, Table 2) when the inoculations were made on the upper leaf surface. Infection from inoculations on the lower surface was, on the other hand, readily obtained and in most cases it was extremely abundant, hundreds of spots developing on every inoculated leaflet.

TABLE 2.—Results of inoculations of the strawberry with *Mycosphaerella fragariae* on the upper and under surfaces of the leaves

Experiment number	Date	Location	No. plants inoculated		Results	
			Upper	Under	Upper-surface inoculation	Under-surface inoculation
I	2/15/29	Greenhouse	3	3	One spot on each of 2 plants; no infection on the third	10 spots on one plant, 36 on another, and 21 on the third
II	3/11/29	Greenhouse	3	3	No infection	3 spots on one plant, 10 on the second and no infection on third
III	11/19/29	Laboratory	1	1	No infection	15 spots
IV <sup>a</sup>	11/27/29	Greenhouse	3	2	8 spots on one plant, 2 on another, and 12 on the third	35 spots on one plant and over 100 on the second
V	1/9/30	Greenhouse	2	2	No infection	No infection
Check <sup>b</sup>	1/9/30	Greenhouse	2	2	No infection	No infection
VI	2/6/30	Greenhouse	3	3	No infection	Hundreds of spots on one plant; no infection on the other two
		Outdoors	2	2	No infection	16 spots on one plant; no infection on the other
VII <sup>c</sup>	3/3/30	Greenhouse	2	2	No infection in 20 days	Hundreds of spots on all the older leaves in 4 days. No infection on younger leaves in 20 days
		Outdoors	2	1	No infection in 20 days	Hundreds of spots on all older leaves in 4 days. No infection on younger leaves in 20 days
VIII <sup>a</sup>	4/19/30	Greenhouse	3	2	No infection	Hundreds of spots in 4 days
		Greenhouse	2	1	No infection	Hundreds of spots in 4 days
IX <sup>c</sup>	11/19/30	Laboratory	1	1	Six spots	Hundreds of spots, coalescing and killing the leaves
X <sup>e</sup>	1/7/31	Laboratory	2 leaves (same plant)	2 leaves	No infection	Very numerous spots
XI	12/4/31	Outdoors	4	4	Spots only around insect holes in leaves (Fig. 2, C and D)	Spots very numerous on all inoculated leaves. (Fig. 2, C and D)

<sup>a</sup> Plants sprayed with water and placed under bell jars 7 hours before inoculating.<sup>b</sup> Sterile water.<sup>c</sup> The plants used in this test were those that showed no infection in experiment VI. (See explanation in text, p. 623.)<sup>d</sup> Same plants as those used in experiment VII. All the leaves showing infection were removed and the plants were placed under moist bell jars without reinoculation.<sup>e</sup> Seedlings of the variety "Banner."



It is considered necessary to explain the abnormal results of experiments VII and VIII (Table 2), in which spots became visible in the short period of 4 days. In experiment VII, the plants employed were those that showed no infection in experiment VI (3 greenhouse and 2 outdoor upper-surface-inoculated and 2 greenhouse and 1 outdoor lower-surface-inoculated plants). Since these plants showed no signs of infection in 25 days, the inoculations were considered ineffectual. Accordingly, the plants were reinoculated, as shown in table 2, on March 3, 1930, and placed under moist bell jars for 3 days. On the fourth day, very numerous spots appeared on the older leaves of the plants inoculated on the lower surface. These spots increased in size during the next few days and eventually became typical of this disease. Four days is too short a period for infection to take place and become externally manifest (the incubation period for strawberry leaf spot is usually 10 to 14 days). The most probable explanation, in the writer's opinion, is that infection had taken place from the first inoculation (February 6) and that the fungus lay in the tissue in a more or less dormant state and resumed growth and became externally manifest when conditions (high humidity) for it became favorable. Additional evidence in support of this explanation is the fact that the spots appeared in 4 days only on the outer leaves, those that were included in both the first and the second inoculations, but not on the young leaves, which were not included in the first inoculation. To test this supposition further, the same plants were used in experiment VIII. In this experiment, all the outer leaves (those included in both inoculations) and all of the younger ones that showed any signs of infection were removed, and the plants, without being reinoculated, were placed under moist bell jars for 3 days. Again, on the fourth day, very numerous spots appeared on all the leaves that had been previously inoculated on the lower surface. No infection occurred on those that had been inoculated on the upper surface.

Dudley (3) reports a somewhat analogous case of dormancy of the *Mycosphaerella* fungus in the tissue. He states (p. 175): "In the early spring, it was found that wholly new spots would appear on diseased leaves, in so short a time after they were brought into the laboratory that no infection could have taken place from newly formed conidia. Probably these diseased areas arose from a fresh growth of mycelium that had hibernated in the leaf."

#### HISTOLOGICAL STUDIES

In general, the same methods described for *Diplocarpon* were followed in investigating the mode of infection of the leaf-spot fungus. Ingress in the case of *Mycosphaerella* was found to be wholly stomatal (Fig. 1). In all the material examined, no indication that the fungus enters by penetration of either the upper or the lower epidermis was ever observed. If this

mode of ingress did occur, it was not found during the progress of these investigations. On the other hand, entrance through the stomata was observed very frequently (Fig. 1, A, B, C, D, F, and H). The germ tube is of about the same thickness as the mycelium of this fungus and in the early stages stains very deeply. It grows and ultimately reaches a stoma. After entering the stoma it may curve and continue its growth between the mesophyll cells, (Fig. 1, C and F), or, often, it may grow straight upward between the palisade cells (Fig. 1, D) toward the upper epidermis. No haustoria or intracellular mycelia were found.

Assuming that the stomata of the strawberry occur only on the under epidermis, it was puzzling at first to reconcile the occasional upper-surface infection (Table 2) with the observed fact that ingress is stomatal. It was soon observed, however, that in the Klondike variety, at least, stomata are not confined to the lower leaf surface alone; they are present also on the upper surface (Fig. 1, E and G), although in much smaller number (Table 3) than on the lower surface. While no actual entrance through an upper-epidermis stoma was observed, germinating spores were often seen in the vicinity of such stomata, and it is believed that the occasional infection obtained from upper-surface inoculations occurred through stomata. Figure 1, G, shows a germinating spore next to an upper-surface stoma, the germ tube curving toward the stomatal opening.

#### STOMATA AND VARIETAL RESISTANCE

Having determined that infection with the leaf-spot fungus is stomatal and that the variety studied (Klondike) possessed stomata on both leaf surfaces, the thought of determining whether or not there was a correlation between number of stomata and varietal susceptibility to this disease suggested itself. In June, 1931, while visiting the strawberry-breeding farm at the United States Plant Field Station, Glenn Dale, Maryland, the writer had opportunity to collect leaf material from resistant and susceptible varieties for study. In this variety plot, where leaf spot was abundant, some varieties were found very severely spotted, while others, growing alongside diseased ones, were disease-free. The latter were considered resistant. The material was collected and placed in killing solution on the spot and later imbedded in paraffin. Some of this material, unfortunately, was lost when the temperature control of the box in which the material was kept got out of order and the paraffin blocks melted, but new material, partly replacing that that was lost, was received through the kindness of G. F. Darrow in October, 1932.

Table 3 summarizes the findings. The figures may not be absolute, and are probably high, because the stomata were counted on cross sections of the leaf, cut 8-10 $\mu$  thick; and so, if a stoma were cut in two, it would

TABLE 3.—Comparison of the average number of stomata per square millimeter of leaf surface of strawberry varieties resistant or susceptible to *Mycosphaerella fragariae*

Variety	Type	Total leaf area examined <sup>a</sup>	Average number of stomata per sq. mm.	
			Upper	Under
Klondike .....	Slightly resistant	36.81 sq. mm.	5.54	265.6
Howard 17 × 633 .....	Resistant	51.14	0	237.7 +
Missionary × ? .....	Very susceptible	39.40	0	302.5
Klondike × 817 .....	Susceptible	14.58	0	233.6 +
N. Y. Station No. 4626	Very susceptible	20.80	0	287.0
Howard <sup>17</sup> .....	Very resistant	32.14	0 <sup>b</sup>	304.7

<sup>a</sup> Sum of many samples taken from several different leaves.<sup>b</sup> Only one stoma found on entire 32.14-mm. area examined.

appear on 2 successive sections and would be counted twice. However, since the same method was used in all cases, the figures are satisfactory for comparison. The results obtained do not indicate that there is a direct correlation between the number of stomata in a variety and resistance or susceptibility to leaf spot. Actually, there was more variation found between different leaves of one variety, or even between portions of the same leaf, than between the average figures of resistant and susceptible varieties.

## SUMMARY

Inoculation tests with *Diplocarpon earliana* (Ell. & Ev.) Wolf and *Mycosphaerella fragariae* (Tul.) Lindau showed that infection with both organisms takes place, predominantly, if not wholly, through the lower leaf surface. The variety Klondike was mainly used in these tests.

Histological studies showed that in the case of *Diplocarpon*, the germ tube enters by direct penetration between the epidermal cells. It was never observed to enter through the stomata. No subcuticular mycelium was ever observed. After the germ tube reaches the mesophyll layer, it branches and the intercellular mycelium grows between the mesophyll cells for some time before branch hyphae reach the palisade cells and the upper epidermis. Haustoria were found. These were more abundant in the peduncle than in the leaf cells.

In the case of *Mycosphaerella*, ingress takes place through the stomata. In no case was it ever found to be other than stomatal. The Klondike variety was found to have stomata on the upper epidermis, also, but they

were decidedly fewer than on the lower surface.

No correlation was found between the number of stomata and susceptibility or resistance to leaf-spot infection in 6 varieties studied.

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# THE TRAP CROP AS A MEANS OF REDUCING ROOT-KNOT-NEMATODE INFESTATION<sup>1</sup>

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The use of the trap crop as a means of contributing to the control of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey<sup>2</sup> has never, in recent years, received serious consideration. Theoretically, the method is sound. In connection with a long series of experiments conducted by the nematology department of the Pineapple Experiment Station in Hawaii, designed to test every known method of nematode control, several experiments on the trap-crop method were carried through. An account of one series, in cans and tubs of soil, is included in this paper. Another series, conducted under conditions more nearly approximating those of the pineapple fields of Oahu, is reported in another paper (13).

## LITERATURE REVIEW

The trap-crop (Fangpflanzen) idea as applied to the control of the plant-parasitic nematodes originated with Kühn who, as early as 1881 (22), experimented with it as a means of reducing soil infestations of *Heterodera schachtii* in sugar beets in Germany. He continued to work with it up to 1886, when he reported at length (23) on a series of several years' experiments. He demonstrated a high degree of efficiency in control with summer rape, *Brassica rapa* L., as a trap crop, and was able to show marked improvement in sugar-beet growth and yield following his treatments. In a much later paper (24) his enthusiasm for the method as a practicable one for field application still holds, and in 1909, according to Müller and Molz (28), he presents a scholarly review of the method and its possibilities.

Several other European students of the sugar-beet-nematode problem make favorable mention of the trap-crop idea without adding materially to it. Among these are Ritzema Bos (30), Fream (9), Marcinowski (25), Blunck (4), and several others, references to whose papers and to the progress reports of Kühn through several years are to be found in the valuable bibliography on the genus *Heterodera*, issuing from the Imperial Bureau of Agricultural Parasitology in England (20). Spiegler (32) states that he has obtained marked results from it in reduced nematodes and

<sup>1</sup> Published with the approval of the Director as Technical Paper No. 60 of the Experiment Station of the Association of Hawaiian Pineapple Cannerys, University of Hawaii.

<sup>2</sup> According to the international code of zoological nomenclature this is the correct name for the root-knot nematode, which has long been erroneously called *Heterodera radicola*. See papers by Goodey (18) and Tyler (34).

increased sugar-beet yields, but that he has abandoned the method because of the danger, in field practice, of actually increasing nematodes rather than reducing them and because of the expense involved in the treatment. Müller and Molz (28) contribute to the trap-crop idea the thought of destroying trap crops at the right stage of growth by means of a poison spray rather than by mechanical means. They present convincing data of decrease in nematode population and increase in crop yield. Molz (27) later practically dismisses the trap-crop method from practical consideration, along with other methods of direct control, with the statement "Praktisch wertlos war das Kühn'sch Fangpflanzenverfahren . . .," and refers with greater hopefulness to the method of stimulating the hatching of eggs with various stimulating substances, developed in part by himself (26) and by Baunacke, Rensch, and Nebel, to whom he refers. Various of these investigators, who worked mostly in Germany, mention the facts of failure to kill larvae that have recently entered roots, and the continued development of some of those that entered earlier to the egg-producing stage if the plants were killed only slightly too late.

There is no record available to us of any investigations in America on the trap-crop method for sugar-beet-nematode control. Shaw (31) reviews the work of the European investigators up to 1915, quoting Spiegler (32) in particular. He appears inclined to agree with Spiegler and discards the trap crop from further consideration for the control of the sugar-beet nematode in America. Other American contributors on the control of this nematode follow Shaw in offering no encouragement for the trap-crop idea.

The early developmental stages of the root-knot nematode, *H. marioni* are very similar to those of *Heterodera schachtii*. Both penetrate completely to the interior of young roots and become established in the tissues where they remain fixed until maturity. No doubt, because of these similarities, there is considerable mention in the literature of the trap-crop method as a possible means of control of the gall-forming nematode. Frank (8) lists it as one of the possible means of control, without experimental support. Cobb (5, 6) in Australia quotes Kühn's method in detail and considers it favorably as a control measure for the root-knot nematode but reports no experimental work on it. Atkinson (1) mentions the trap-crop method of control. Stone and Smith (33) list it along with other methods of control, quoting the European investigators Kühn and Hollrung as authorities. They contribute no original work on the method. Bessey (2) cites the European investigations and considers their possible application for root knot nematode control. He conducted experiments in the southern States in America for 2 years with varying results. Since treated plots and check plots did not show noticeable differences in succeeding crops, some of the plants remaining free, some slightly affected, and some very badly affected by root

knot, Bessey decides adversely as to the value of the trap-crop method. He and Byars (3) do not include the method in their list of recommendations for nematode control in their *Farmers' Bulletin* on the subject, nor does Godfrey (10) in his. Godfrey (11, p. 246) reports a great reduction in nematode content of soils used a second time, after a first test in which plants were removed before the propagation of a new generation of nematodes. Godfrey and Morita (14) reported briefly on a phase of the studies included in the present paper, in an Academy of Science Proceedings not widely distributed. This paper is cited because of its listing in the general Heterodera bibliography (20) previously mentioned. The present paper gives a much more complete report. No other investigator of the root-knot problem in America contributes any new information on the trap-crop method.

#### BASIC CONSIDERATIONS

The theory of the use of the trap crop for trapping this nematode from the soil is based on the fact that, once established inside the root, the larvae of *H. marioni* are incapable of further motility. Not until the males are fully mature do they make their final moult from the fusiform body wall and migrate to the exterior or to another position within the root. The female does not move away from her fixed position at all. This is fully elucidated in an earlier paper from this Station (16), in one by Nagakura (29) in Japan, and elsewhere. The original larvae, then, are virtually imprisoned within the roots and are removed from all possibility of parasitizing another plant. It is only their progeny that can bring about new infection. The trap-crop idea, then is simply to plant a highly susceptible crop in the soil and to "catch" as many nematodes as possible in the root system. Before the stage of development is attained at which a new generation will develop, the plants are plowed under or otherwise killed and the roots die, resulting in the death of the contained nematodes. The roots need not necessarily even be removed from the soil so long as all possibility of continued growth is eliminated. The rate of reproduction of this nematode is so rapid that only a few new colonies, started by the development and hatching of egg masses, would quickly overcome the benefit derived from the trapping of the original population. The enclosed immature females must not have attained that critical stage where they will continue to develop and produce eggs, even if the host plant roots are not functioning. The element of time of destruction of the host plant, therefore, is a very important one in connection with the use of the trap crop.

There are peculiar difficulties incident to studies on soil populations of this nematode that have thus far made it impossible, by any known method of taking a soil census, to account for all the nematodes present in a soil

at any particular time. Another paper (12) deals at length with this subject. In the experiments herein recorded this fact becomes particularly evident. Differences in soil type, soil moisture, season, condition of the nematodes, natural enemies, host-plant growth, and other factors controlled with difficulty, make for lack of uniformity in results in different lots of soil receiving supposedly identical treatments. For any one experiment these factors are made as uniform as possible except for the controlled variables in all lots. Different experiments, however, are probably not strictly comparable.

#### EXPERIMENTS

The experimental work reported in this paper comprises 4 experiments conducted from 1926 to 1932.

##### A Test of Period of Growth in Relation to Trapping, with the Tomato as a Trap Crop

*Experiment 1.* This experiment, started in August, 1926, had 2 objectives, the first to determine the length of life cycle of the root-knot nematode under Hawaiian conditions, and second, to determine the practicability of the trap-crop idea. Tomato plants were grown in flats of sterilized soil and later transplanted to small pots to promote good root growth. In the meantime soil heavily infested with nematodes was thoroughly mixed and placed in a series of about 30 1-gal. cans. When the tomato plants were well established they were transplanted, without disturbing the root systems, into the centers of the gallon cans, and growing conditions were maintained. The tomato plants were in such condition that rapid development of good root systems took place. According to plan, 3 plants were removed on every third day, the roots washed thoroughly to free them from soil, and examined and records were made of the number of galls and of stage of development, according to the indicator-plant method reported in another paper (12). Following this 2 separate procedures were followed. (1) A second planting of tomatoes was made in the same soils from which the first 3 plants had been removed. This second planting remained in the soil in every case only until just before the egg producing stage of the parasite, as indicated by observations on separate plantings. At this time they were removed and still another planting made. This was continued until galls were no longer found. In this way, no reproduction occurring in the soil, each successive planting reduced the nematode count of the soil more and more. The gall count indicated rate of reduction. (2) The roots removed from the soil in every case were carefully washed to remove all possibility of surface infection and were used to inoculate pots of sterilized soil. Into these pots tomato plants previously started in sterile soil were planted. By this means it was possible to determine to what extent nematodes capable of causing new infection were carried over inside the roots.



The results in numbers of knots on the roots of tomato plants in 4 successive plantings in the same soils, are indicated in table 1. The roots of the tomato plants in lot 1, a, b and c, showed no galls when removed 3 days after inoculation. Obviously this was too short a period for the larvae to find the roots or, if infection did occur, for galls to have become evident. The nematodes left in the soil infected the second plant. This explains the greater abundance of root galls in the second planting in this case. When

TABLE 1.—*The effect on subsequent infection of periodic trapping by removal of infected roots from soil badly infested with Heterodera marioni*

Lot number (All inoculated 9/17/26)	1st planting			Subsequent plantings (all 24 days)					
	Roots removed after (days)	No. of galls	Av.	2nd planting		3rd planting		4th planting	
				No. of galls	Av.	No. of galls	Av.	No. of galls	Av.
1 a	3	0		6		37		0	
b	3	0		103		14		0	
c	3	0	0	44	51	15	22	0	0
2 a	6	23		3		12		0	
b	6	85		35		8		0	
c	6	29	46	2	13	3	8	0	0
3 a	9	203		0		86		0	
b	9	111		0		0		16	
c	9	298	204	3	1	4	30	0	5
4 a	12	493		42		9		0	
b	12	503		0		57		0	
c	12	587	528	0	14	3	23	0	0
5 a	15	1236		10		7		3	
b	15	1011		7		0		0	
c	15	2153	1467	11	9	2	3	0	1
6 a	18	1316		0		81		27	
b	18	2855		0		19		0	
c	18	3903	2691	0	0	8	36	0	9
7 a	21	5000		0		1		0	
b	21	4020		0		2		0	
c	21	2097	3706	0	0	8	4	42	14
8 a	24	3000		13		0		0	
b	24	1200		11		5		45	
c	24	5000	3067	15	13	23	9	3	16
9 a	27	*6092		107		3		0	
10 a	28	**4030		83		13		0	
11 a	29	**6500		56		76		0	
12 a	30	**6500		21		47		3	
13 a	31	**5000		3000		44		2	
14 a	32	**4500		7		57		0	

\* Egg masses first found.

\*\* Egg masses abundant.

one considers the low total number of galls for this first lot in the entire 4 plantings, however, it would seem either that high mortality had occurred in the soil, or that a large number of nematodes must have entered the roots without visible symptoms and must have died with the roots when they were removed. The same situation exists for the one or two subsequent lot numbers.

Numbers of galls in the first plantings increased up to the 27th day. This was to have been expected both because of the increased root system with the passage of time, which means simply that more roots are available for infection, and because of the greater prominence of galls. Egg masses were first found on the lot-9 plants, removed 27 days after inoculation. Even here, as well as in the next 3 days, the careful removal of the roots carried most of these egg masses with them, as is shown by the low infections in the second plantings. This shows that in their early development the egg masses adhere very firmly to the roots and likewise that as yet no hatching of eggs has occurred in the soil. At 31 days either the egg masses were easily dislodged or some hatching had already occurred in the soil, for a sudden sharp rise in the amount of infection in the second planting occurs in this lot. The absence of this rise in the lot-14 plants is an irregularity in results that might be accounted for by a soil-texture difference (possibly less water), which permitted the more complete removal of root systems or of a decline in the early hatching of eggs.

In every case after lot 1 there is a decline in gall count in the second planting which becomes more pronounced, proportionately, as the original gall count increases. In most cases this decline is a very abrupt one. It is evident that the majority of nematodes were caught in the first planting. It is likewise evident, however, that not all the nematodes were caught even in the third successive planting, and possibly not even in the fourth. The reasons for this are clear. The original inoculating material consisted in part of undecayed roots containing unhatched eggs and confined larvae which were not released into the soil until after one or more plantings had been removed. That this is an important factor in increasing the longevity of nematodes in the soil is shown by Godfrey, Oliveira, and Gittel (17), Jones (21), and Guba (19). Again, larvae in the soil do not always find their way to roots, even during long periods of exposure of root systems to infection. We have evidence from experiments and observations that the chemotactic movement of larvae through soil to susceptible plant roots is not the all-important factor in infection. Rather, susceptible roots must be growing near where the nematodes are originally located for infection to take place. This point is mentioned again elsewhere in this paper. Müller and Molz (28) show that the same is true for *Heterodera schachtii*.

Figure 1 shows graphically the great difference between the first and

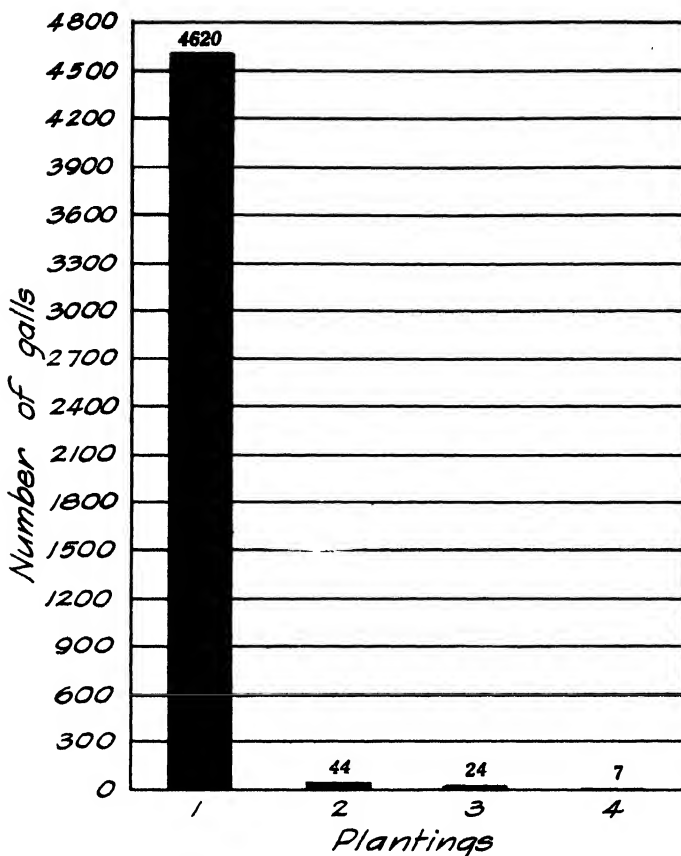


FIG. 1. Graphic representation of the results of experiment 1 in nematode catch, as recorded by count of galls produced, in 4 successive plantings of tomatoes as trap crops. The 4 columns depict the average readings on lots 8 to 12 (see Table 1).

subsequent plantings in number of galls produced on indicator plants up to the time when marked propagation of a new generation of nematodes is evident. This chart is made up of the average gall counts from lots 8 to 12. Over 98 per cent of the total number of galls produced in all 4 plantings occurred in the first. Lot 13, in which increase had occurred, would again show a high reading for the second planting.

Table 2 shows the results of the second phase of the trapping test, made by using the roots removed from the first planting for inoculating new cans of sterilized soil. The inoculum for plant 1 was made up of the entire root systems of plants a, b, and c in lot 1; that for plant 2 of lot 2, a, b, and c, etc. The results are what might have been expected, that is, very low transfer of infection up to the 24th day and then a sudden rise on the 27th day when egg masses were transferred with the roots. The same results are

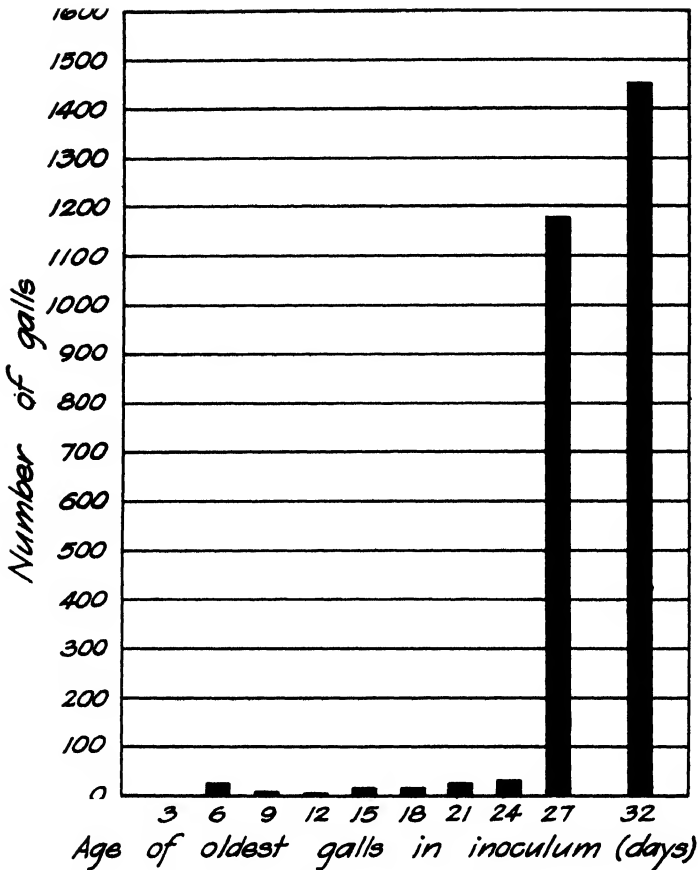


FIG. 2. Graphic representation of the amount of infection in tomato roots grown in soils inoculated with infected roots of different ages. See text for complete explanation and table 2 for exact figures.

depicted graphically in figure 2. The transfer that does occur in the earlier stages was obviously due to very recent infections in the roots in which the larvae, not yet established in the roots, were still capable of moving out into the soil again where they were available for infection of the new plants. The gradual increase in numbers of such infective larvae that were transferred, up to the 24th day, might be expected because of the daily increase in number of susceptible root tips available for new infections. The same situation exists with *Heterodera schachtii*, as reported by Müller and Molz (28) and other European investigators of the trap crop for that nematode.

This experiment demonstrates a very heavy reduction in nematode population from a single planting of a trap crop. It likewise demon-

TABLE 2.—*Results of use of root galls of different ages as inoculum on root knot in subsequent plants. (Roots examined in every case after 30 days)*

Plants	Source of inoculum (See Table 1)	Age of oldest galls in inoculum	No. of galls devel- oped on test plant
1 .....	Lot 1 a, b, c	3	0
2 .....	Lot 2 a, b, c	6	24
3 .....	Lot 3 a, b, c	9	9
4 .....	Lot 4 a, b, c	12	7
5 .....	Lot 5 a, b, c	15	17
6 .....	Lot 6 a, b, c	18	18
7 .....	Lot 7 a, b, c	21	27
8 .....	Lot 8 a, b, c	24	32
9 .....	Lot 9 a	27	1,177
10 .....	Lot 10 a	32	1,453

strates, however, that several trap-crop plantings are necessary in order to entirely eradicate nematodes from the soil. Even with the confined roots in the gallon cans used in this experiment not all the nematodes in 3 plantings and possibly not even in four were removed. The negative readings in most cases on the 4th planting would seem to indicate complete eradication. This conclusion is not completely justified, however, since in some cases negative readings in the third planting were followed by positive readings in the fourth. (Table 1).

#### Preliminary Tests of the Cowpea (*Vigna Sinensis*) as a Trap Crop in Soils of Known Initial Nematode Population

*Experiments 2 and 3.* In the previous experiment the initial nematode population of the soil was unknown. It was therefore possible to determine only the relative quantities of nematodes removed from the soil in each of the several plantings of trap crops. No means were provided for determining the absolute numbers of nematodes removed by each plant or the relationship between total numbers of nematodes removed and the original population.

Other experiments were conducted in which efforts were made to determine these relationships. In 2 such experiments, in which cowpeas were grown in gallon cans of soil and root-observation boxes (7), with known initial magnitudes of infestation, poor plant growth occurred, probably because steam-sterilized soil was used in order to eliminate chance nematode contaminations. The roots did not penetrate the soils so well as did similar plantings in the field. Consequently results were irregular and unsatisfactory, and this paper is not encumbered with a detailed account of the tests. Certain consistent conclusions are of interest, and serve to support similar conclusions from a final comprehensive test. They are incorporated in the conclusions therefrom.

### Known Nematode Populations in 5-Gallon Containers of Soil Freed from Chance Contamination by Thorough Air Drying

*Experiment 4.* The final experiment of this series, installed primarily in connection with the studies on indicator crops reported elsewhere (12) and described fully therein, is included here only in so far as its results are pertinent to the trap-crop problem. Two series of 5-gallon (20-liter) jars consisting of 20 jars each were filled with soils previously freed from living *Heterodera* by thorough air drying (15) and inoculated with nematodes, 1 series with 50 larvae to each jar, and the other with 500. Cowpeas were planted and, on sprouting, were thinned to 1 plant per jar. Growth of the plants was good. The readings, on the entire root systems carefully removed after 30 days' growth, in numbers of galls and actual nematode content as determined by the clearing method (16), are summarized in table 3. This experiment was highly satisfactory in that it determined, with sufficient replications and low probable errors, the proportion of a known original population caught by a single planting of a trap crop under good growing conditions in a large volume of soil (approaching field conditions in range of root growth).

TABLE 3.—*The gall count and nematode catch in a single trap-crop planting in 5-gallon containers with known initial population*

Lot	Initial population	Nematode catch (Average of 20 plants)		Percentage catch
		Gall count	Nematode count	
A .. . . .	50	10.55 $\pm$ .40	11.10 $\pm$ .41	22.2
B . . . . .	500	131.8 $\pm$ 4.73	150.35 $\pm$ 7.63	30.07

### DISCUSSION AND CONCLUSIONS

In the first experiment the heavy root systems of transplanted tomatoes were responsible for the better than 98 per cent catch of the total effective nematode population in the first of a series of 4 successive plantings. In 2 subsequent experiments cowpeas were seeded in containers of steam-sterilized soil inoculated with known numbers of nematodes, and obviously good conditions for plant growth were sacrificed to certainty of control of the population. Root growth was poor and clearly unable to reach the nematodes, and the results obtained are discarded. In a final test this difficulty was overcome by the use of sun-dried rather than steam-sterilized soil, which permitted of equally accurate nematode population control and at the same time good plant growth. In this case catches of 22 to 30 per cent of the original population were obtained in the original planting. The actual proportion of the "effective" population caught, however, may be

considered to be much higher than that indicated by these figures. This conclusion is based upon the results obtained from all the tests in which subsequent plantings were made (Experiments 1, 2, and 3) in which invariably all the later plantings have, together, caught much fewer than the original planting. The remaining nematodes, not accounted for in the root examinations of trap crops, may be considered to be in 1 or more of the following categories:

1. Those that have not found roots and that may do so later if a more extensive root system of a susceptible crop becomes available.
2. Those that have found roots but have failed to become established.
3. Those that have perished in the soil because of initial inherent weakness or some long-continued unfavorable condition of the environment (15, 17).

The tests indicate that class 1 accounts for only a small proportion, and it may be concluded that many larvae perish without producing infection.

The following conclusions are drawn from the trap-crop experiments: (1) A very high proportion, up to 98 per cent, of the total "effective" nematode population of soils in closed containers was caught by a first planting of a vigorous trap crop with a heavy root system, penetrating well through the soil. (2) Only from 22 to 30 per cent of total original populations were so caught in the first plantings, but the uncaught remaining populations were mostly "ineffective" in producing infection in subsequent plantings. (From tests 2 and 3 in part). (3) A considerably higher proportion of a higher population was effective in gaining entrance into root systems, than of a lower initial population, indicating some joint activity of larvae in penetrating roots (see 16, p. 328). This conclusion was consistent in tests 2 and 3 as well as in test 4. (4) In tests in which the larvae used for inoculation were placed in definite regions to one side of the container (test 3) only the roots in that immediate vicinity became infected, indicating a minimum migration of the larvae through the soil.

#### SUMMARY

The trap-crop (Fangpflanzen) method of reducing populations of plant-parasitizing nematodes, which during the past 50 years has received a great deal of attention in Europe in connection with the sugar-beet nematode, *Heterodera schachtii*, has occasionally been suggested for the control of *H. marioni*, but never has been adequately tested. Preliminary trap-crop tests in containers of limited capacity, in Hawaii, have shown that nematode populations can be very greatly reduced by one or more trap-crop plantings. Well-rooted tomato plants set in gallon cans of heavily infested soil and removed before the development of a new generation of nematodes, withdrew from the soil over 98 per cent of its effective nematode

population in a single planting. The roots removed from the infested soil and used to inoculate new cans of sterilized soil transmitted very low numbers of infective larvae up to the 27th day (under the conditions of the experiment), at which time heavy transmission took place due to the abundance of eggs at this stage of development. The limitation of root systems in this test to gallon cans undoubtedly made for a more complete catch than would occur in open fields. In another test cowpeas were planted as trap crops in 5-gallon containers of originally nematode-free (air-dried soils) inoculated with known numbers of nematodes. Twenty-two per cent were recovered with a single planting in the case of the low population inoculation (50 larvae), and 30 per cent in the high-inoculation series (500 larvae). In other similar tests, subsequent plantings of trap crops recovered only low additional proportions, showing that most of the uncaught larvae after first plantings were ineffective in producing infection. In the most intensive test, however, the third successive planting of a trap crop did not entirely eradicate the nematodes, and the fourth did not do so with absolute certainty, though remaining infestation was decidedly low.

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# A STUDY OF THE ROOT-KNOT-NEMATODE TRAP CROP UNDER FIELD SOIL CONDITIONS<sup>1</sup>

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## INTRODUCTION

A companion paper from this Station<sup>2</sup> gives the results of greenhouse experiments with tomatoes and cowpeas as trap crops in small containers of soil. A review of the literature on the use of a trap crop for nematode control is given in that paper. A very high percentage reduction in nematode populations was obtained under the conditions of one experiment in a single planting of the trap crop. This was, of course, removed from the soil before the development of a new generation of nematodes. In 3 subsequent plantings the final ones still showed in some cases the presence of infestation.

In an effort to determine if, under conditions more nearly approximating those of the field, the trap-crop method might have a practical application, a further series of experiments was started at the Wahiawa field headquarters of this Station in the spring of 1930. Observations from a field rotation-crop experiment had shown, following 2 plantings of a trap crop, a very great reduction in nematode population of the soil as demonstrated by a later planting of an indicator crop. This reduction was clearly sufficient to be of significance in connection with the growing of a short-season annual crop. Its value for a perennial crop such as pineapple, was questionable, however, in view of later observations on the experiment referred to, which is discussed elsewhere in this paper. The great reduction mentioned from the 2 plantings, however, was encouraging as to the value of further intensive studies on the trap-crop proposition.

## METHOD

A main desideratum of these new experiments was to approach field conditions as closely as possible in so far as type and depth of soil were concerned and yet to have the factors of uncertainty as to spread from one location to another completely eliminated.

This was accomplished by means of "isolated plots" like those used by

<sup>1</sup> Published with the approval of the Director as Technical Paper No. 61 of the Experiment Station of the Association of Hawaiian Pineapple Cannerys, University of Hawaii.

<sup>2</sup> Godfrey, G. H., and Helene M. Hoshino. The trap crop as a means of reducing root-knot-nematode infestation. *Phytopath.* 24: 635-647. 1934.

Byars<sup>3</sup> in Florida in his work with poisons. These consisted of 4 x 8 ft. galvanized iron bottomless boxes with sides 3 ft. deep inserted into the soil to a depth of 2 ft. The boxes were arranged end to end in rows, a single partition serving to separate adjacent boxes. They were riveted together at the corners, which were well painted with white lead to eliminate possible migration of nematodes from one box to another. The 2-ft partition below ground is practical insurance that no movement of nematodes from one box to another takes place in that region. The 1-ft. elevation of the sides above ground eliminates danger of surface spread by splashing, etc. The top soil was 10 inches deep. The final level of soil on the inside was approximately 2 inches on the average above that on the outside.

The area in which these boxes were placed had stood for years in elephant grass, *Pennisetum purpureus* Schumacher, a plant that is apparently immune from root knot. A test by means of indicator crops showed the initial nematode populations to be uniformly low. This presence of nematodes was probably due to the few susceptible weeds that grew in the field along with the grass.

The first step in the plan of procedure was to inoculate all plots for the experiments uniformly and heavily with the root-knot nematode. This was accomplished by inserting a uniform application of heavily infested cowpea roots. This being shown by test to be short of the heavy degree of initial infestation desired, additional inoculum was applied to each plot in the form of about 2 bushels of heavily infested soil from pineapple fields and the whole spaded under.

The main objective in the experiments to be conducted in these plots was, of course, to reduce the nematode populations of the plots as quickly as possible to zero, and at the same time to learn the utmost of what actually happens in the roots of susceptible plants when the latter are killed at different stages of nematode development. The proper time for removal of the plants was, naturally, an important consideration. The influence of season on length of life history of the nematode was recognized but at the time the experiments were installed the minimum length of life cycle of the nematode was not known.

*Experiment 1:* For this experiment it was decided to treat different plots as follows:

*A Plots* In duplicate. Susceptible crop (cowpea, *Vigna sinensis* L.) planted six rows in the plot, rows 6 inches apart, plants 4 to 6 inches apart in the row. At end of "x" days plants destroyed by turning under into the soil. In doing this a few individual roots were marked by means of strings and labels for later ob-

<sup>3</sup> Byars, L. P. Experiments on the control of the root-knot nematode, *Heterodera radicicola* (Greef) Mueller. Phytopath. 9: 93-103. 1919.

servation on the development of nematodes in decaying roots in the soil.

*B Plots* In duplicate. At the end of "x" days the plants were removed from the soil by so pulling as to get the major portion of the root systems. In this way it was expected that the comparison between A and B methods of plant destruction could be obtained. A few of the B roots were then thoroughly washed and placed in moist chambers for intensive study of further development of the contained nematodes.

*C Plots* In duplicate. Plants removed at end of "x + 1" days. Procedure otherwise same as with A.

*D Plots* Growing plants were left in the ground continuously. They were turned under at maturity and new plantings made; thus a continuous high nematode population could normally be expected.

*E Plots* Kept continuously fallow until eradication appeared to be complete in A, B, and C, when a crop was planted for purposes of comparison.

The first planting was made on April 10, 1930, and left, according to plan, for 23 days. It soon became evident that this period was too long, since laboratory examination of roots removed showed the development of egg masses. Such roots turned back into the soil would naturally constitute reinfestation. The succeeding few plantings were devoted to an effort to determine the maximum length of time plants could be left in the soil without bringing about an actual increase in the nematode population. The results of these efforts are shown in very compact form in the first part of table 1. The last column but one of this table shows the results in presence or absence of eggs from the laboratory examination of roots removed according to plan under the outline for A and B. It is obvious from the table that up to the end of the 6th planting, which was made on September 4, 1930, some reinfestation of the soil was taking place from roots that were returned to the soil.

Beginning on October 7, the plantings were continued, this time with assurance that plants would be removed in time to prevent such reinfestation. Special plantings of the same indicator plant in infested soil, made on the same dates as the trap-crop plantings, removed daily for examination near the approximate date, governed time of removal of the latter. An increase in length of cycle can be considered as evidence that, on the average, the soil was cooler than when the cycle was shorter. In other words, seasonal change in length of life cycle is taken into consideration. Observations were recorded only occasionally after this date, as shown by the continuation of table 1. On January 12, 1931, only 6 per cent of the plants

TABLE 1.—*A record of observations on trap-crop experiment No. 1*

Cycle	Date planted	Plots	Plants growing (days)	Infection			Development of eggs on roots after incubation period in	
				No. plants	Inf.	Eggs	Laboratory moist chambers	Indicator plants from the plots
	<b>1930</b>				<i>Per cent</i>			
1	4-10	A	23		100	—	++	
		B	23		100	—	++	
2	5-22	A	21		?	+++	++	+
		B	21		?	+++	++	
3	6-20	A, B,	19			+	+	+
		C	20			+	+	+++
4	7-16	A, B,	17				+	
		C	18				+	
5	8-11	A & B	15		+		—	+++
		C	16		+			+++
6	9-4	A & B	15					+
		C	16					+
7	10-7	A & B	20		+			
		C	21		+			
8	11-5	A & B	21		+			
		C	22					
9	12-9	A & B	21		+			
		C	22		+			
10	<b>1931</b>							
	1-12	A	28	235	6			
		B	28	236	2			
		C	29					
		D	29		100			+++
		E	60					+
Continued through 12 more cycles, in length as follows: 11th, 24 days; 12th, 21; 13th, 18; 14th, 14; 15th, 13; 16th, 15; 17th, 16; 18th, 15; 19th, 18; 20th, 21; 21st, 28; 22nd, 21. Observations as follows:								
13	4-28	A	18	140	0			
		B	18	203	0			
		C	19	244	0			
14	5-29	A	14	182	14.3			
		B	14	183	13.7			
		C	15	202	8.4			
18	9-8	A	15	138	2.2			
		B	15	165	1.8			
		C	15	212	6.6			
22	<b>1932</b>							
	1-11	A	21	831	0			
		B	21	1618	0			
		C	21	1512	0			
		E	21	113	0			
23	2-18	A	60	92	1 plant	+		
		B	60	90	0			
		C	60	93	0			

removed from A plots showed infection and only 2 per cent of those from B. On this same date plantings were made in plots D and E. D plots showed very heavy infection, as might have been expected. E plots showed

only the slightest trace and that only after 60 days growth of the indicator plants. On April 28, 1931, in the 13th planting from the start of the experiment, but only the 5th after reinfestation ceased, the readings on all plants were zero. However, in readings subsequent to that, infection of plants began to appear again. It was not until the 22nd planting from the start, or the 16th after reinfestation ceased, that the readings on all plants were zero again. This zero reading was on January 11, 1932, approximately 14 months after reinfestation of the plots theoretically ceased.

In the entire tabulation the absence of a record means simply that no observations were recorded. Negative readings are not implied. After the second reading from the start of the experiment, infection was invariably very light, in most cases only 1 or 2 galls on each plant that was recorded as positive.

As a final check on this experiment another replanting was made on February 18, 1932, this to be left for 60 days. On April 18, 1932, these plants were removed for observation. Only a single plant in the entire lot, this in plot A, showed a single gall with one female and an egg mass. Thus, after 15½ months of trap cropping with 16 successive plantings, at least one of the plots still retained infestation.

*Experiment 2:* An entirely different series of trap-crop plantings was made in another set of isolated plots starting on June 3, 1931. All plots were inoculated at the outset, by the addition of heavily infested pineapple soil. The soil was likewise enriched with decomposed pineapple waste to render conditions favorable for good plant growth. There were 3 treatments similar to those of Experiment 1, all in duplicate.

*A Plots* Plants turned under at end of "x" days.

*B Plots* Plants removed with major part of root system at "x" days.

*C Plots* Plants turned under at "x + 1" days.

The length of the cycles were determined in part by previous experience on the basis of influence of season on length of life cycle, and, in part, by stage of development of galls on roots, as determined by observations on occasional plants.

The lengths of successive cycles, in days, were as follows: 1st, 14; 2nd, 18; 3rd, 14; 4th, 14; 5th, 15; 6th, 20; 7th, 21; 8th, 21; 9th, 23. With C plots the cycles were, of course, a day longer. It will be noted that here, too, the low-temperature cycles were of greater length in order to trap as many nematodes as possible before the first ones to infect the roots reached maturity. Notes on extent of root-knot development were taken on only a few of the successive trap-crop plantings, so observations are not tabulated. There were 9 successive plantings of trap crops. The first showed definite and relatively uniform but not extremely heavy infection. Subsequent observations showed only a trace; toward the end of the series only

occasional plants showed any infection, at all. The only observations recorded during the course of the series were as follows: 8th cycle, 22 days, ending January 4, 1932, C plots showed one plant only infected; 9th cycle, 23 days ending February 10, 1932, negative throughout.

Following this regular cycle, the entire series was replanted and left for 60 days. This gave time for deeper penetration of root systems to depths in the soil not reached by the shorter cycle plantings and likewise time for reinfestation from the progeny from any chance primary infection that may have occurred. The readings on this again showed a trace of infection in 4 of the 6 plots in the test. Two of these were from B plots and 2 from C plots, the A plots being zero throughout. Thus 9 successive plantings made through 8½ months did not completely eradicate the nematodes from the soil in most cases.

#### MISCELLANEOUS TRAP-CROP RESULTS FROM OTHER EXPERIMENTS

*The Contribution of Trap Crops to Nematode Control in a Field Culture Experiment.* During the period 1927 to 1930 a 4-acre pineapple field at the Wahiawa Field Station that was heavily infested with root-knot nematodes in the 1927 plant crop, was made available for a series of different intercycle culture operations with the objective in view of testing them both for effects on the nematode population of the soil and on pineapple yield in the planting to follow. The area was divided into 8 equal plots each measuring 75 by 300 feet, to receive 4 duplicated sets of treatments, A to D. Without explaining the experiment as a whole in detail, a trap-crop phase of one portion of it is here described because of its bearing on the subject-matter of this paper.

Two plots, C and D, both duplicated, had virtually the same set of treatments including, in order, a winter of a cover crop, a summer of clean fallow, another winter of cover crop and summer fallow, then nematode-resistant winter-cover crop again, and finally spring plowing and continuous clean fallow in preparation for fall planting (1930). The one outstanding difference between the 2 treatments was, for C plots, 2 plantings, in February and March, 1929, of English peas as a trap crop, one closely following the other. Both plantings were watched closely, with frequent examinations of roots, and were plowed under before time for the propagation of a new generation of nematodes. In February, 1930, the winter cover crop of pigeon peas, *Cajanus cajan* [L.] Millsp., growing in both C and D plots, was noticed to have harbored a heavy growth of weeds throughout the winter. The weeds in D plots known to be nematode-susceptible (principally *Emilia sonchifolia* L.) were abundantly, uniformly, and almost 100 per cent infected with nematodes. In C plots the same weeds were only scatteringly infected (about 10 per cent). Some

propagation of nematodes undoubtedly occurred in both sets of plots, since the weeds were growing a sufficiently long time to bring about such increase. Approximately the same order of difference in infestation persisted, however, throughout the balance of the preparation-for-planting period. An indicator planting of English peas in April showed a uniform, heavy infection for D plots. Another planting made at the same time in tubs containing composite soil samples from the different plots showed definite infection for D plots and no infection for 1 C plot with a bare trace for the other. The field was planted in November, 1930, to pineapples, according to regular plantation practice. Soil samples collected from the different plots the latter part of the same month and planted to cowpeas as indicators showed definite infestation present in both C and D plots, but distinctly greater in the latter. Final nematode observations were made on English peas planted in large numbers at regular intervals beside the pineapple rows in January and February, 1931, and removed for root observations about a month after planting. A summary of the results of this reading is given in table 2. A significantly lower degree of soil infestation is still manifest in the C plots 2 years after the trap crops were used.

TABLE 2.—*Summary of indicator crop readings of C plots (with trap crop) and D plots (without)*

Plot	Number of plants		Plants infected
	Infected	Not infected	
C-1 .....	175	3166	<i>per cent</i> 5.2
D-1 .....	1358	1704	44.3
C-2 .....	394	3288	10.7
D-2 .....	1719	1888	47.6

Consideration of all the results in nematode readings on this experiment would appear to justify the conclusion that the trap-crop plantings definitely contributed to nematode reduction. Since there was nematode survival of a relatively low order throughout, however, it is not likely that the difference brought about by the trap crop is sufficient to make itself felt significantly in the pineapple planting. Unfortunately, insufficient experiments have been conducted with single-season crops to form an adequate basis for judgment as to the value of the extent of reduction obtained when such crops come into consideration. We have only the general information from the indicator-crop studies previously referred to (Ref. 12, other paper), that a high percentage of indicator plants showing infection means



a relatively high degree of soil infestation, and one that will make itself manifest on the growth of a susceptible crop planted immediately; and on the other hand, that a low percentage of such plants showing infection means relatively low infestation of the soil and in all probability one that will permit such a crop to grow through a season without serious injury. Further experiments are needed on the common susceptible annual crops, both to determine the amount of injury obtained from known initial infestations and to determine the practicability of the use of the trap crop to reduce infestation to noninjurious proportions.

*Spraying with a Weed Killer to Destroy Trap Crops.* In July, 1930, 5 tubs of soil heavily infested with nematodes were planted to cowpeas. Seventeen days after planting, the plants in 3 of the tubs were sprayed with sodium arsenite, containing 5 pounds arsenic to 100 gallons. The spray was completely effective in killing the aerial portions of the plants. A week later the nonsprayed plants showed continued development of the nematodes within the roots, and egg masses with eggs were evident. In the sprayed plants such continuation of development did not occur. Nematodes could be found within the roots in the early pyriform stage, without sign of egg masses. In another week roots were well decayed. The spraying had effectively killed the plants and had definitely resulted in the destruction of the nematodes that were caught in the roots and prevented the propagation of a new generation. Repetitions of the experiment produced the same result. Observational sprayings on weeds and indicator plants under field conditions likewise brought about efficient killing of the plants.

These indications of the practicability of poison sprays in connection with the use of trap crops are in line with the contributions of Müller and Molz in Europe, referred to in the other paper (Ref. 28). The work with poison sprays for killing trap crops, planned for large-scale field application, was not continued owing to the press of other activities. A study of the extensive literature on weed killing indicates, however, that a number of different sprays would be entirely practicable for the purpose in mind, should trap crops be used for nematode reduction on any large scale.

*An Attempt to Reduce Nematode Population in the Pineapple Row by Trap-crop Plantings.* In 1927 and 1928 susceptible soybeans and cowpeas were planted close beside the rows of extremely heavily nematode-infected young pineapple plants with a view to determining if repeated planting and removal of such trap plants would reduce the extent of pineapple infestation. A first planting of soybeans, made in late August, was extremely heavily infected, and each plant definitely removed from the soil thousands of nematodes. Thereafter throughout the winter and until June of the following year repeat plantings were made along the same rows, and removed always before the maturation of the nematodes. Each planting was

compared in degree of infection with a first planting in a near-by row. Until the sixth planting no marked differences were evident, practically all plants alike showing medium or heavier infection. From that time on until the 11th and final planting of the test the repeat plantings showed some reduction in degree of infection as compared with the first plantings; but, even in the last planting, infection was definitely present in most of the plants. Table 3 shows the course of the nematode readings obtained throughout the test, in terms of index figures representing degrees of infection, from 0 to 5. Since relative infections only are relevant, the exact number of galls for each index figure is not necessary, though roughly, the number 5 may be considered to represent about 1,000 galls per plant and each digit below that about 200 less.

TABLE 3.—*A record of degree of nematode infection on a series of trap-crop plantings beside the pineapple row. (Scale 0 to 5)*

Plantings	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th	11th
"Repeat" plantings	5	3	4	3-5	4	2-3	1		2	2	0-4
"First" plantings for comparison		5	4	5	5	2-3	1-5	...	4	4	0-5

Pineapple plants removed at random from the rows receiving the influence of the 11 trap-crop plantings showed no visible reduction in nematode infection as compared with near-by rows, and no effect whatever on plant growth was evident. It would appear that the soil around the pineapple plants had already, at the start of the test, reached the "saturated" condition in so far as nematodes were concerned, and that only a surplus of nematodes that might otherwise largely have perished were caught in the trap plants from the limited range of soil reached by their roots. The nematodes in the pineapple root systems were constantly propagating new generations and thereby maintaining a supply sufficient to infect the new roots as fast as they entered the ground. The trap crop in this case was apparently of no value to the pineapple plants.

#### DISCUSSION

The detailed observations on the stage of development of nematodes at the time of removal or destruction of trap plants in the first experiment herein reported make it evident that the presence or absence of the initiation of an egg mass is not a safe criterion on which to base judgment as to the chances for further propagation of nematodes if the roots are left in the soil, even when they are detached from the plants. In the course of development of the female there would appear to be a critical point in the

early pyriform stage before which it probably will die if the plant is killed and after which it may eventually attain maturity and deposit at least a few eggs. Miss Tyler, in her pure culture studies (Previous paper, reference 34, p. 378) has shown that the nutritional requirements of the nematode may be met without a growing top to the plant at any stage of nematode development. She has carried the nematode through its complete life cycle, from infection of the root to maturity and egg development, in small detached roots. The decay of the roots, then, before eggs are produced, must be considered as the ultimate factor that prevents continued development and the propagation of a new generation. From this necessary point of view, conditions favoring the rapid decay of the roots of the trap plants are an important adjunct to the successful use of the trap-crop method. This is a soil-management problem, and involves the assurance of the presence of sufficient soil bacteria of the right sort, and of nitrogen and phosphorus, and moisture and temperature conditions favorable for microbiological activity.

The conclusion is obvious from this series of tests that the trap crop may be of value only in quickly reducing heavy soil infestation to a relatively low point. It is decidedly not indicated for nematode eradication. Under field conditions the roots do not ramify through the deeper layers of soil, where the organisms may be expected to survive longest, sufficiently well during the limited period that is allowable, to catch them all in the 2 or 3 plantings that would be practicable. Older plants, 60 days or more in the ground, will reach more of them, but this period of growth is so long that the earlier infections are almost certain to produce a new generation of larvae and thus defeat the purpose of the trap crop. While the use of trap crops has been unpromising as an *eradication* measure, it has definitely brought about high percentage *reduction* of nematode population in 1 or 2 plantings only. This feature of the results of these trap-crop studies, if judiciously applied, may prove to be of practical value *in connection with* other means of nematode control.

#### SUMMARY

Further studies with trap crops for the reduction of soil population of the root-knot nematode, *Heterodera marioni* (Cornu), were conducted under conditions approaching those of the field. The plots used for the experiments were of normal field depth and were separated from one another, to avoid cross contamination, by means of galvanized iron sheets 2 feet in the ground and 1 foot aboveground. In 2 tests, cowpeas (*Vigna sinensis* L.) and English peas, *Lathyrus sativus* L., both highly susceptible to root knot, were planted as trap crops in a succession of several plantings. In the trapping tests proper they were removed from the ground or

turned completely under, always before the development of a new generation of nematodes. Thus, every planting destroyed some of the original nematode population. In neither of the tests was a final zero-point infestation reached in all plots, even after, in the one case, 16 successive plantings during nearly as many months, and in the other case 9 successive plantings. A bare trace of survival occurred in both cases. Nematodes in the deeper layers of soil were not reached by the roots during the short period of growth (14 to 24 days, depending upon the temperature) necessary to insure that no reproduction would take place. No better results in final eradication were obtained by this method than were obtained by plots maintained under completely fallow conditions during the same period. For complete eradication, therefore, the trap crop is not to be considered. Two plantings, however, reduced infestation by a high percentage (upwards of 90 per cent), so that, judging by general knowledge of the growth of plants in nematode-infested soils, a susceptible annual would survive the season without serious injury.

A test in a heavily infested young pineapple field gave substantial proof that a long succession of plantings of trap crops beside the crop row was not capable of reducing the nematode population sufficiently to become obvious in the pineapple plants. Other tests indicated the practicability of the use of poison sprays for the killing of trap crops at the right stage, under field conditions.

# IS PSOROSIS OF CITRUS A VIRUS DISEASE?<sup>1</sup>

H. S. FAWCETT<sup>2</sup>

(Accepted for publication August 21, 1933)

A symptom of psorosis expressing itself as a mosaic-like effect on young leaves has recently been discovered (6). This, taken in conjunction with information that has been accumulating for a number of years, suggests the probability that it is a virus disease. It is the purpose of this paper to present the results of experiments and observations that bear on this phase of the subject. More evidence is needed to prove beyond question the virus nature of the trouble.

The histology of tissue affected with psorosis, as compared to normal tissue, will form the subject of another publication by Irma E. Webber and the writer.

The characteristic effects of psorosis heretofore observed on the trunk and branches have been described in detail in previous publications (3, 4). In brief, one of the first evidences on the bark is either small scales of bark pushed up from the surface and attached by one edge, or small, pimple-like pustules or eruptions (Fig. 1). These usually start at some small localized region and enlarge slowly to form large areas. It is not uncommon for only one area to be seen on the trunk or large limb at first. Frequently, however, a number of small areas will become visible at the same time, and these by enlargement and merging form large areas encircling the trunk or a large limb in one season. Drops of gum are later exuded.

Another symptom (5) first makes its appearance as a drop or drops of exuded gum, then the bark begins to scale in much the same manner as the other, but usually with a more rapid spread and a more rapid continuous extension along branches to smaller and smaller twigs. Later, effects of both forms show continued scaling of outer bark and gum formation, not only in the bark elements, but also in the region of the wood. Frequently, the final effect after some years is a staining of the deeper layers of wood in the trunk and larger limbs and either a slow or rapid decline of the tree.

The hypothesis that the causal agent of psorosis is a microscopic organism, such as a fungus or bacterium, has directed investigation of this disease for many years. Such a theory of the disease had appeared logical because of the definite, localized scaly bark lesions. These start, in the beginning stages, in the outermost layers of bark and spread slowly in surrounding tissue, and only later affect the deeper tissues of the bark, cam-

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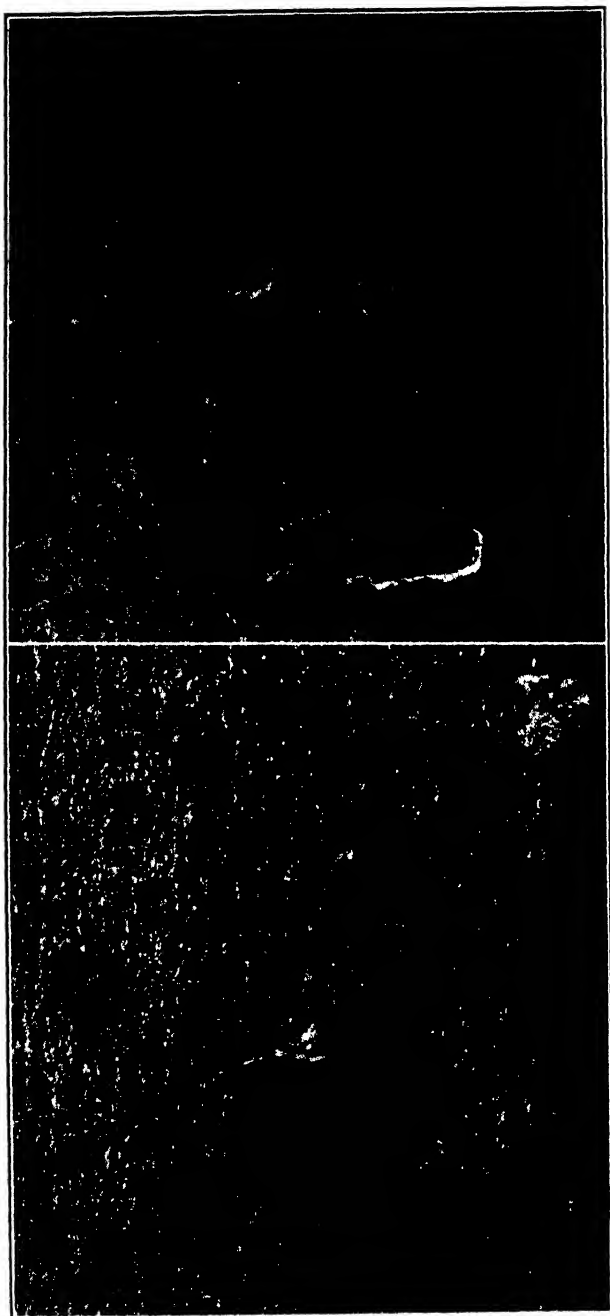


FIG. 1. Beginning stage of psorosis on the bark, showing formation of scales pushed up from the surface. About  $\times 1$ .

bium, and wood. Moreover, the manner of development of these lesions is not unlike that of other bark diseases, the causal agent of which was known. In fact, shell bark of lemon (2), now known to be due to the fungus *Phomopsis citri*, is somewhat analogous to psorosis in the peeling off, in scales, of outer layers of bark, while the inner bark and cambium remain alive. In a few instances, 4 cases out of 100 or more trials, psorosis has been transmitted to healthy orange trees by placing under the bark pieces of tissue from diseased lesions. In these cases 2 to 8 years elapsed before the expression of the disease was noted on the bark of the trees inoculated. In an attempt to determine the causative agent, many species of organisms have been isolated in past years from the superficial tissues of psorosis bark lesions, but inoculations have all failed to reproduce the disease.

The symptoms associated with psorosis, which have not been previously described in detail, are mosaic-like effects on young leaves and clear, more or less circular spots on older mature leaves, spots on water sprouts, and rarely on fruit.

#### Mosaic-Like Effects on Young Leaves

This condition was noticed for the first time on May 15, 1933, at Riverside, California, on the same Valencia orange trees from which buds had been taken for propagation in 1922, as described later. The mosaic-like appearance is found to be produced by many small, elongated, light-colored areas usually in the region of the smallest veinlets of the leaf blade. The effect appears to be due not only to a clearing of the veinlets themselves, but to a clearing in tissue just adjacent to the veinlets, as well (Fig. 2). These small, cleared places usually are very numerous and either scattered over the entire blade or occupy only certain portions of it. When pronounced, the effect gives a stippled appearance to the small young leaf blades when held to the light. As the leaves become larger and approach maturity, the stippled effect is masked. The areas that produce the stippled or mosaic-like effect vary much in size. Spots are often about 1 to 3 mm. long and  $\frac{1}{2}$  to 1 mm. broad. Sometimes they are about the same length and width, and, when only 1 mm. or smaller in diameter, give the appearance to the unaided eye of being more or less circular dots. Often the pattern is not distinct and the spots fade out gradually at the margins or run together. In very small young leaves an appearance of vein-clearing alone is sometimes seen. They appear to be more pronounced in young leaves on rapidly-growing shoots or water sprouts than on leaves of slower-growing shoots.<sup>3</sup>

<sup>3</sup> Since this article was submitted, attention has been called to a mosaic-like variegation of sour orange leaves in Sicily, described by L. Petri, who suggests that it is transmitted by an aphid *Toxoptera aurantii*. Judging from Petri's illustration and description, it is believed to be different from the mosaic-like symptom of psorosis in California

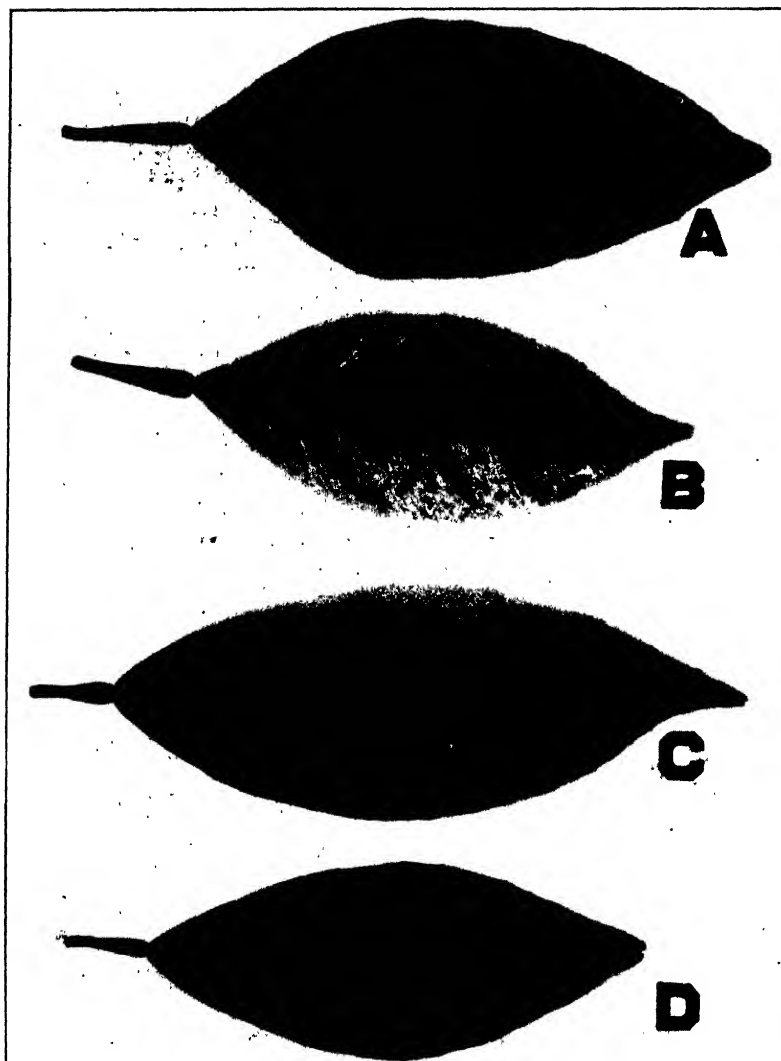


FIG. 2. Immature leaves of Valencia orange. A and B. Mosaic-like symptoms by transmitted light. C and D. Healthy leaves. About  $\times 1\frac{1}{2}$ . Photographed by L. J. Klotz.

#### Spots on Older Leaves, Water Sprouts, and Rarely on Fruit

The spots on older leaves that had been noted occasionally for some years past vary in size and appearance from mere dots to larger, translucent sweet orange. Unlike the mosaic of psorosis, the one described by Petri is accompanied by definite warping and crinkling of the leaves and appears to depend on the previous presence of aphids on the leaves affected.

Petri, L. *Variegatura infettiva delle foglie di "Citrus vulgaris" Risso*. Boll. R. Staz. Patol. Vegetale 11: 105-114. 1931.



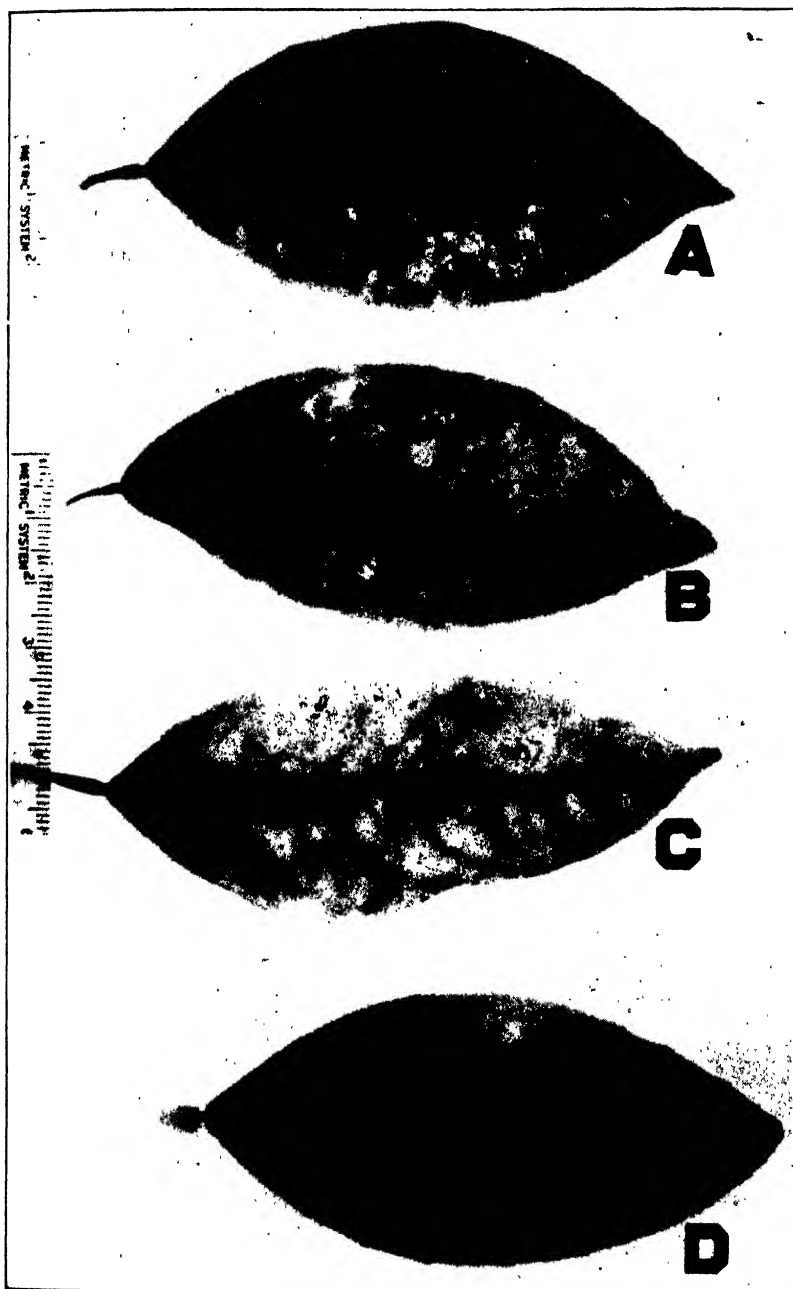


FIG. 3. Mature leaves of Valencia orange with light spots associated with psorosis. In A and C raised corky rings show in the light areas. About  $\times 2$ . Photographed by L. J. Klotz.

cent areas more or less circular and frequently in the form of rings (Fig. 3). Corky, raised areas, forming either a pustule in the center, or rings or partial rings, are common in these spots. This raised, darker appearance is produced by greater cell division below and deposition of certain products in some of the layers of cells near the surface. Few or many such spots occur on a single leaf.

Although these spots vary greatly in size and appearance, the following actual examples may be mentioned. Some Valencia orange leaves on trees with psorosis bark lesions showed, in July, circular to irregular, yellowish, translucent spots  $\frac{1}{2}$  to 7 mm. across, not raised, with indefinite margins, scattered over the blade. Others showed spots 1 to 6 mm. in diameter, with raised, corky dots  $\frac{1}{2}$  mm., in the center on the upper surface. Other leaves had pronounced irregular ring spots 5 to 10 mm. in diameter. One area 8 mm. in diameter had 2 corky concentric rings, the smallest 1 to 2 mm. in diameter, and the largest 4 mm. with a yellow halo beyond. The rings are often not complete.

The spots that occur occasionally on the bark of water sprouts that originate from a badly diseased trunk or large limb resemble at first, the raised spots on mature leaves, and occur with or without rings. Later, as the water sprout matures, the raised portions may become more corky, glazed, and hard, and in some cases resemble the spots so commonly produced by the presence of leprosis (4). On only three different occasions has any effect on fruits been noted that might have a connection with psorosis. One of them was 2 or 3 grapefruits with depressed, circular furrows borne on a branch affected with psorosis. Another was a similar, less pronounced circular furrow seen recently on a single Valencia orange fruit on a tree badly diseased. A third was a Navel orange fruit with a dark-brown circular band about 10 mm. in diameter on a tree with an extremely bad and old case of psorosis. These fruit effects, if symptoms of psorosis, appear to be quite rare. Usually all fruits on badly diseased trees appear to be normal.

Following a careful study of these symptoms on both young and old leaves, it has been possible to find them either in a pronounced or inconspicuous form in association with a large majority of badly affected cases of psorosis on both Valencia and Navel orange trees. However, many trees with psorosis on the bark in a less severe form have been examined with no visible leaf symptoms.

It is believed that psorosis may occur on the bark either as a localized form, without being systemic until it has been on the tree for a number of years, or that it may be transmitted by budding in a systemic form and express itself on the bark after the tree is 6 or more years of age.

This hypothesis of systemic and localized forms is based not only on the

observations mentioned above but on the results of treatment of bark lesions in their beginning stages. Many trees appear to have been cured of psorosis by either scraping off the outer affected bark or by cutting away the bark over the diseased portion. On other trees the disease appears to have been eliminated by cutting off a certain branch that was first affected. At present, however, the data are not sufficient to establish with certainty this hypothesis.

#### Transmission by Buds

On April 28, 1922, bud sticks consisting of small twigs just back of fruit were taken from 5 Valencia orange trees on which there were bark lesions farther back on the larger limbs or on the trunk. The twigs used as a source for budding were not visibly affected with bark lesions. If leaf symptoms were present they were entirely overlooked.

A similar set of bud sticks was taken from five other trees that at the time showed no visible bark lesions of psorosis. Three of these 5 trees, however, afterwards developed bark lesions, leaving only 2 on May 15, 1933, that were free from psorosis. Buds from all of these were placed in sour orange nursery trees on May 3, 1922. The trees were set out in orchard form on May 5, 1924. They were examined from time to time and no differences were noted until the summer of 1931 when some trees began to show one or more small inconspicuous lesions on the bark of the trunk or older branches. This was 9 years from time of budding.

The mosaic-like symptoms had not been discovered at that time, so no data as to this effect are available. On May 15, 1933, the mosaic-like symptoms were discovered on the parent trees having psorosis. The next day a survey of the experimental plot gave the results shown in table 1.

It will be noted that all of the trees originated by buds from trees with visible psorosis in 1922 showed the mosaic-like symptoms in 1933 on young

TABLE 1.—*Experiments in transmission of psorosis of Citrus by budding*

Condition of parent trees	Number of trees propagated	Number of trees on which symptoms were apparent on May 16, 1933		
		On young, immature leaves	On mature leaves	On bark
Psorosis, 1922 .....	23	23	20	14
Psorosis, 1932 .....				
No psorosis, 1922 .....	17	9	6	4
Psorosis, 1932 .....				
No psorosis, 1922 .....	11	0	0	1
No psorosis, 1932 .....				

leaves, and 14 out of 23 trees showed symptoms on the bark. It is believed that the parent trees that showed psorosis in 1922 had the systemic form and, therefore, transmitted the disease through the buds, and that all of the progeny will eventually show it on the bark. In most cases of psorosis in commercial orchards the bark lesions are not apparent until the trees have been set out for from 8 to 10 years, and usually even longer.

About one-half, or 9 out of 17, of the progeny from the parent trees that showed no psorosis on the bark in 1922, but had developed bark symptoms by 1932, show the mosaic-like leaf symptoms. Four out of these 9 show the bark symptoms. It is not known when this group of parent trees first showed bark symptoms, but the extent of the diseased areas in 1932 indicated that the symptoms had been present for a long time. If psorosis was carried by the buds, at least part of these buds may have been permeated in 1922 by the psorosis-producing principle, even though no bark symptoms were then evident on these parent trees.

None of the trees originating from the 2 parent trees free from visible psorosis in 1922 and still free in 1932 showed any mosaic-like leaf symptoms or mature leaf symptoms in May, 1933. One tree, however, in this group showed bark symptoms. It is believed that this tree may not have had the systemic form and may have become infected from the outside since it was budded. This would agree with the hypothesis that the disease may exist either as localized lesions and only later becoming systemic, or that it may be systemic from the first by being transmitted by budding from a parent tree that already had the systemic form.

#### Experiments with Rooted Cuttings

In December, 1926, F. F. Halma rooted some leafy shoots from a Valencia tree about 20 years old, showing a severe case of psorosis on the older bark. The bark lesions had been noted about 7 years before, when the tree was about 13 years old. The shoots themselves appeared to be healthy and nothing abnormal was noted at the time. Other shoots from healthy trees were rooted at the same time. These were set out as small trees in 1929. In June, 1933, the trees originating from a psorosis-infected tree showed the mosaic-like symptoms on young, immature leaves, and clear spots on older leaves, but no bark symptoms. Trees originating by rooted cuttings from a healthy tree in the same orchard were free from these effects. The same symptoms on leaves were found on young Navel trees propagated in March, 1928, by means of rooted leafy shoots taken from a tree badly affected with psorosis, while those similarly propagated by means of leafy cuttings from healthy trees showed no symptoms.

Since the discovery of the mosaic-like symptom, a series of experiments has been started on the basis of the virus hypothesis to determine more

definitely the mode of transmission and development of the disease. Inoculation experiments in collaboration with L. J. Klotz and experiments in budding, bark grafting, etc., have been initiated. Having learned to recognize the mosaic-like symptom, it has already been possible to recognize after only 5 weeks the symptom on the new shoots that have grown out from the diseased buds placed in healthy nursery stock.

The general resemblance of some of the symptoms of psorosis (especially the effects frequently found on mature leaves and water sprouts) to leprosis and ring-blotch lesions suggest the possibility that these may be in the same class of diseases. The nature of the cause of neither of the latter two diseases has so far been satisfactorily explained. E. M. Doidge (1) has previously suggested, among other hypotheses, the possibility of a virus for the ring blotch in South Africa.

#### SUMMARY

A symptom of psorosis appearing as a mosaic-like effect on young leaves was discovered in May, 1933. This is found most distinctly on young leaves of rapidly growing shoots. The mosaic-like effect is produced by small, light-colored areas in the region of the smallest veinlets. Symptoms on the mature leaves are light-colored, translucent spots often with raised, corky centers or with raised rings. Frequently, raised spots are found on the bark of water sprouts. Circular bands or furrows on fruit, probably associated with psorosis, have only rarely been found.

The previously well-known conspicuous symptom, which was evidenced by localized areas of scaly bark, had heretofore led to the hypothesis that the cause should be sought for in a microscopic organism.

The finding of these mosaic-like effects on leaves, together with the transmission of the disease by budding and by rooting leafy shoots, suggests a virus origin for the disease.

Experimental and observational evidence is given for the suggested hypothesis that the disease may occur either in a localized form restricted to a portion of the bark or in a systemic form spread throughout the tree to the buds, leaves, and other parts. It is suggested because of the general character of some of the lesions of psorosis on leaves and water sprouts to those of leprosis and ring blotch that these three diseases, though distinct, may belong to the same class of diseases.

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# THE ASSOCIATION OF CERCOSPORELLA HERPOTRICHOIDES WITH THE FESTUCA CONSOCIATION<sup>1</sup>

RODERICK SPRAGUE

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## INTRODUCTION

*Cercospora herpotrichoides* Fron is a soil-borne fungus, which causes a foot rot of winter wheat, *Triticum vulgare* Vill., and winter barley, *Hordeum vulgare* L., in certain prairie areas in Washington, Oregon, and Idaho (2, 3, 4, 5, 6). While there is small likelihood that the disease will become general throughout the wheat-growing sections of these States, the fungus causes severe losses in certain areas. The following data on its ecological associations are presented.

## THE NATIVE VEGETATION OF CERCOSPORELLA-INFESTED AREAS

The vegetation of the prairie regions in the Columbia Basin largely belongs to what Weaver (7) termed the Agropyron-Festuca association. He divided this association into two consociations, the semi-arid bunch grass or Agropyron (dominant) consociation on the one hand and the more humid region sod-grass or Festuca (dominant) consociation on the other. Weaver's type area was Whitman County, Washington; the dry western portion belonging to the Agropyron consociation, and the somewhat moister eastern half to the heavier sod-grass or Festuca consociation.

Range stock and the plow have made heavy inroads on the native vegetation of the Cercospora-infested localities, but there remain enough unmolested portions to determine the native flora with a fair degree of accuracy. Most of the Cercospora foot rot occurs on Festuca sod-grass prairies (Festuca consociation) very similar to those studied by Weaver, except that most of the soils are somewhat lighter than those of the type area in the Palouse Region of Whitman and adjacent counties. A discussion of the native flora of the several Cercospora-infested areas follows.

## Columbia Gorge Area

*Tygh Ridge, Wasco County, Oregon.*—The Cercospora-infested fields lie on a rolling, dissected plateau country, just east of the conifer-covered Cascade range, at an elevation of 2,700 feet, and within sight of the Columbia Gorge. Scattering trees and shrubs occur on the slopes below the wheat fields, but unmolested portions of the hill tops are covered with a

<sup>1</sup> Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon and Washington Agricultural Experiment Stations.

*Festuca* sod-grass consociation. In this consociation *Festuca idahoensis* Elmer (dominant),<sup>2</sup> *Poa sandbergii* Vasey, and *Agropyron* spp. are the more prominent grasses. In the drier locations clumps of *Agropyron spicatum* (Pursh) Scribn. and Smith are more prominent, and on rocky outcrops occur still more primitive, scattered grass associates. In sod-grass bordering foot-rot-infested wheat fields certain spring flowering plants occur in considerable numbers. Among these *Tellima tenella* (Nutt.) Walp., *Lomatium geyeri* (S. Wats.) Coulter and Rose, *Fritillaria pudica* (Pursh) Spreng., and *Brodiaea grandiflora* Smith are prominent during the spring months. In late spring and summer *Paeonia brownii* Dougl., *Balsamorhiza deltoidea* Nutt., *Astragalus* spp.,<sup>3</sup> *Lupinus leucophyllus* Dougl., *L. laxiflorus* Dougl., *Eriogonum compositum* Dougl., *E. sphaerocephalum* Dougl., *E. heracleoides* Nutt. and *Eriophyllum lanatum* (Pursh) Forbes, dominate the flora.<sup>4</sup>

*Seven Mile Hill, Wasco County, Oregon.*—This Cercospora area is on a wind-swept prairie 1,500 feet above the bottom of the Columbia Gorge (fig. 1, A and B). The prairie was originally covered with an ill-defined sod-grass consociation in which the flora was similar to that on Tygh Ridge. Prominent flowering plants growing adjacent to foot-rot-infested fields include *Sisyrinchium douglasii* A. Dietr., *Fritillaria pudica*, *Delphinium menziesii* DC., *Balsamorhiza deltoidea*,<sup>5</sup> *Eriophyllum lanatum*, *Astragalus hoodianus*, *Lupinus caudatus*, Kellogg, *L. leucophyllus* and *L. laxiflorus*. There are thickets of Oregon oak, *Quercus garryana* Dougl., on thin soil portions of the prairie, which are bordered by the following shrubs: *Symphoricarpos racemosus* Michx., *Ceanothus integerrimus* Hook. and Arn., *Opulaster opulifolius* (L.) Kuntze and *Rosa* spp. There also are scattered wind-beaten red-fir trees, *Pseudotsuga mucronata* (Raf.) Sudw., encroaching from the near-by Cascade Range (figs. 1, A and B). Clements (1) illustrated a portion of this prairie as typical of the *Agropyron-Festuca* association.

<sup>2</sup> *Poa sandbergii* is now dominant in moderately grazed areas; *Festuca idahoensis* Elmer and to some extent *Agropyron* spp. disappear under grazing. In over-grazed areas, *Bromus tectorum* L. replaces most of the native grasses. This holds true for Cercospora-infested areas.

<sup>3</sup> *Astragalus hoodianus* Howell is common on High Prairie, *A. conjunctus* S. Wats. on Tygh Ridge, and *A. reventus* A. Gray in the far eastern part of Oregon and Washington.

<sup>4</sup> Phenological observations show that the Cercospora disease on Tygh Ridge becomes evident first as *Lomatium geyeri* comes into bloom; is well started when *Fritillaria pudica* blooms; and, under average seasonal conditions, is advancing most rapidly when the mass of spring flowering plants are making their greatest show.

<sup>5</sup> The *Balsamorhiza deltoidea* of the Columbia Gorge region differs considerably from the typical species west of the Cascades. It may be a transitional species between *B. deltoidea* and *B. sagittata* (Pursh) Nutt.



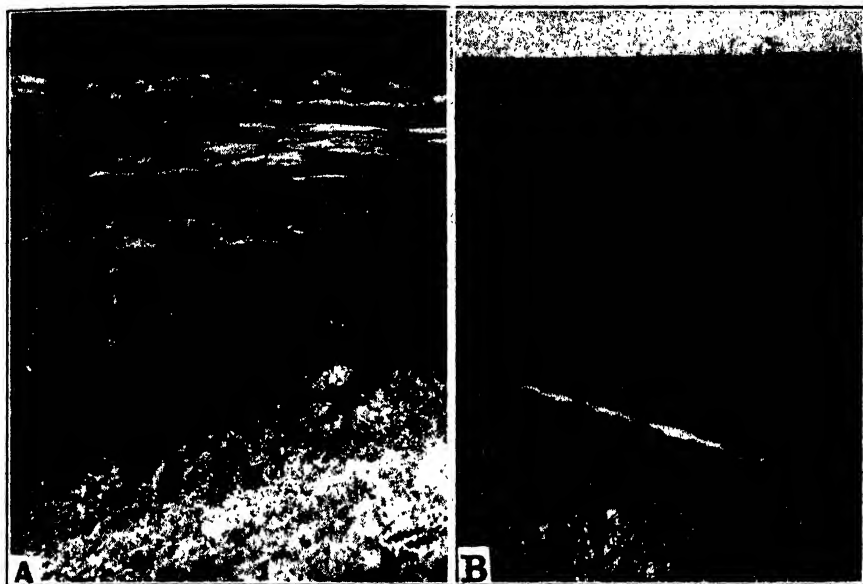


FIG. 1. A. View of Columbia Gorge with sod-grass prairie of Seven Mile Hill, Ore., in foreground and wheat fields on High Prairie, Wash., across the Columbia River in distance. Note directions of prevailing winds (up stream) as indicated by "wind timber" in foreground. Photograph taken with Eastman red-sensitive plate. B. General view of Seven Mile Hill, Ore. Most of the open prairie areas are now seeded to grain. The thickets of Oregon oak with scattered red firs are shown. The edge of the Columbia Gorge is outlined in the distance by "wind timber" red firs.

The *Cercospora* foot rot sometimes occurs in mild form several miles westward from the type area in the transition territory between the grassland and fir-forest climaxes. This territory is characterized by a mixture of deciduous trees and second-growth red fir.

*High Prairie, Klickitat County, Washington.*—This *Agropyron-Festuca* prairie lies at an elevation of 1,600 feet and is a dozen miles, air line, across the Columbia Gorge from Seven Mile Hill (fig. 1, A). The prairie is nearly treeless, the surrounding slopes being covered with trees or bare, depending on exposure. A few miles east, in a treeless region near Goldendale, Washington, a few minor foot-rot infestations occur during average seasons in moist swales of isolated fields.

Most of the original vegetation on High Prairie was of the *Festuca* consociation (fig. 2, A) resembling that of Seven Mile Hill. Scattered spots of a soil phase whiter than the predominating type (possibly subsoil outcrops) and poor in organic matter, are nearly free from foot rot. This type of soil, which, on virgin lands, bears an *Agropyron* consociation, also, produces a poorer crop of grain than do the darker soils of the *Festuca* consociation.

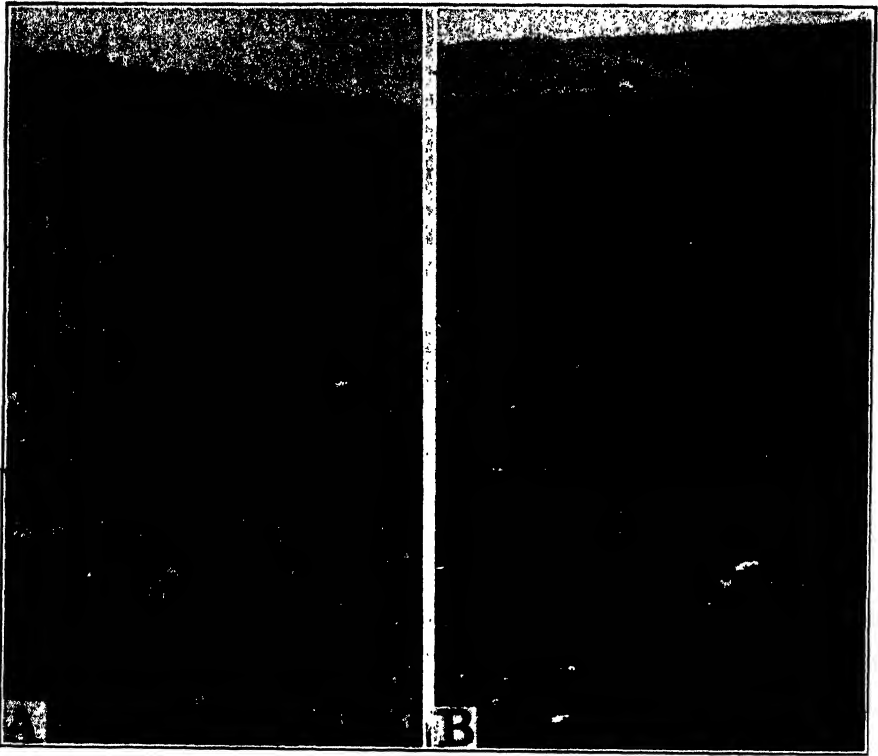


FIG. 2. A. *Balsamorhiza deltoidea* associe in full bloom on High Prairie, Wash. B. Prairie near Liberty Lake Junction, Wash., adjacent to *Cercospora* foot-rot-infested field. Clumps of *B. sagittata* are prominent in the center foreground. *Potentilla*, *Gaillardia*, and *Achillea* also are prominent.

### Grande Ronde Valley Area

This area lies in northeastern Oregon (Union County) about 200 miles east of the Columbia Gorge area. The comparatively level valley, surrounded by the conifer-blanketed Blue Mountains, is 2,800 feet above sea-level. The principal foot-rot-infested area is in the center of the upper valley (Sand Ridge) centering about the towns of Alicel and Imbler. In pioneer days this was rich grassland. Most of the native vegetation has vanished, but various prairie lupines, *Gaillardia aristata* Pursh, *Lithospermum ruderales* Dougl., and *Delphinium menziesii*, typical of the *Festuca* consociation, are still fairly common along roadsides adjacent to foot-rot-infested fields. Moreover, a dozen miles farther up the valley near Elgin, where foot rot also occurs, the low hills adjacent to foot-rot-infested wheat fields are covered with vegetation similar to the *Festuca* consociation of eastern Whitman County, Washington (7). *Balsamorhiza sagittata*,

*Clematis hirsutissima* Pursh, *Sisyrinchium douglasii*, *Brodiaea grandiflora* Smith, *Festuca idahoensis* and *Agropyron spicatum* are present.<sup>9</sup> *Paeonia brownii*, a very restricted species, is reminiscent of Tygh Ridge. *Lupinus sulphureus* Dougl. is the most abundant species of *Lupinus* near Elgin.

#### Anatone Area

This area, which is in Asotin County, Washington, was found during the summer of 1932. It occupies a long strip of territory lying on the Asotin Highlands just below the edge of the Ponderosa<sup>7</sup> pine (*Pinus ponderosa* Dougl.) belt. The elevation is 3,600 feet, the soil a dark, very fine sandy loam,<sup>8</sup> and the vegetation that of a sod-grass prairie. *Balsamorhiza sagittata* and *B. incana* Nutt., *Lupinus laxiflorus*, *Astragalus reventus* and *Lithospermum ruderales* occur. The flora of rocky outcrops is dominated by *Wyethia amplexicaulis* Nutt., *Delphinium menziesii*, and *Phlox viscida* E. Nels. The early spring flora contained *Tellima tenella* and *Lomatium cous*.

#### Spokane Valley Area

The Cercospora foot rot occurs on a considerable acreage in the Spokane River Valley extending from Millwood, Washington, to a point beyond Post Falls, Idaho, and on Peone and Pleasant Prairies, northeast of Hilliard, Washington.<sup>9</sup> The soil of the Cercospora area in the Spokane Valley is Garrison gravelly loam, containing a high percentage of small to midsize gravel. Probably because of the nature of this soil, the grass cover tends to form a bunch-grass type.<sup>10</sup> Characteristic plants are typical of the Festuca consociation (Fig. 2, B). Prominent plants near Liberty Lake Junction, Washington, were *Sisyrinchium douglasii*, *Balsamorhiza sagittata*, *Gaillardia aristata*, *Phlox longifolia* Nutt., *P. rigida* Benth., *Delphinium menziesii*, *Eriophyllum lanatum*, *Lomatium* spp., and *Lupinus sericeus* var. *flexuosus* (Lindl.) C. P. Smith. The original vegetation on the prairies north of the Spokane River was similar to that on the gravelly soil, but was more truly of the Festuca type. On the parts of these prairies where foot rot occurs, the soil is fine sandy loam of several related subtypes

<sup>9</sup> As in other Cercospora areas, *Poa sandbergii* has survived the inroads of grazing animals. *Festuca idahoensis* and *Agropyron spicatum* were found in sheltered places.

<sup>7</sup> Foresters and lumbermen are almost universally agreed on calling *Pinus ponderosa* the Ponderosa pine rather than the older name of western yellow pine.

<sup>8</sup> The soil near Anatone probably represents the same general soil type as the black earth belt of Russia.

<sup>9</sup> Late in the summer of 1932 an area in Spokane County, west of Spangle and Plaza, also was reported. This location has not been surveyed by the writer.

<sup>10</sup> *Agropyron inerme* (Scribn. and Smith) Rydb. and *A. riparium* Scribn. and Smith, together with *Festuca idahoensis*, *Poa sandbergii* and *Koeleria cristata* (L.) Pers. produce a luxuriant growth of grass in unmolested parts along the railroad right-of-way.



(6). All of the foot-rot areas in Spokane County and adjacent Idaho are surrounded by Ponderosa pine forests, which, in turn, merge with other conifer associations as the Rocky Mountains are approached.

The small foot-rot-infested area near Coeur d'Alène, Idaho, occurs on logged-off land that originally was covered with fir and Ponderosa pine. This is one of the few instances where foot rot has "advanced" beyond the adjacent grassland areas.

#### INDICATOR PLANTS

The more prominent native plants that occur in the several foot-rot areas are listed in table 1. Six of these occur generally and may be taken as indicator plants. They are *Festuca idahoensis* (dominant), *Agropyron inerme* and *A. spicatum*, *Balsamorhiza deltoidea* and *B. sagittata*, *Delphinium menziesii*, *Lomatium triternatum* (Pursh) Coult. and Rose, and *Lithospermum ruderales*. All six occur on Seven Mile Hill, Oregon, on Tygh Ridge, and Friend, Oregon, on High Prairie, Washington, near Imbler, Oregon, and in Spokane County, Washington.

*Cercospora* should not be expected to occur in all soils that once harbored these particular plants, since these plants occur over some range of climatic and soil factors. Nor does *Cercospora* entirely restrict itself to the prairie areas where these plants are found. The exceptions to the occurrence of *Cercospora herpotrichoides* in association with this particular plant consociation are so few, however, as to strongly suggest that the *Cercospora* foot rot will remain a more or less localized problem.

#### SUMMARY

The *Cercospora* foot rot occurs almost entirely in prairies that originally bore a *Festuca* sod-grass consociation. Indicator plants are *Festuca idahoensis* (dominant), bunch grass (*Agropyron* spp.), *Balsamorhiza* spp., *Delphinium menziesii*, *Lomatium triternatum*, and *Lithospermum ruderales*.

The writer is deeply indebted to Dr. Helen Gilkey for the checking of seed plant collections made from the foot-rot areas and to Dr. A. G. Johnson for aid in the preparation of the manuscript. Representative specimens of the vegetation of the several *Cercospora* areas are deposited in the Herbarium of the Department of Botany, Oregon State College, Corvallis, Oregon.

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# THE MYRIOGENOSPORA DISEASE OF GRASSES

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(Accepted for publication August 14, 1933)

Clumps of living *Andropogon* received in July, 1931, from Bogalusa, Louisiana, had stunted and twisted shoots, this abnormal condition presumably being due to the fungus, *Myriogenospora*, which was abundantly present. In an attempt to propagate the fungus the diseased grasses were planted in six 6-inch pots and kept in a high temperature greenhouse (70–100° F.) until June, 1932, after which time they were kept out of doors until July, 1933. Although all the clumps survived the transplanting, only one continued to bear the fungus and the diseased condition throughout the season of 1932. In 1933, however, the infection was no longer evident and this clump showed only a healthy condition. The continuance of the infection through 2 seasons has, however, afforded an opportunity to observe a little-known but distinctive pathological effect that seems worthy of record.

Throughout the period from July, 1931, to October, 1932, almost half the clump remained normal without trace of disease, the presence of the fungus being limited to certain shoots that stood out in decided contrast because of their dwarfed and fasciated condition (Fig. 1). The diseased shoots possessed a healthy green coloration and grew vigorously; they were without any chlorotic effect except for the slightly reddish pigmentation immediately adjacent to the stromata of the fungus. On inspection the feature most notable was a characteristic adhering of leaf tips to adjacent leaves or stems that had arisen from a common axis (Fig. 2, A). This attachment seems due chiefly to the mechanical binding by the immature stroma of the fungus during the time the adjacent leaves and culms were still folded and packed together within a subtending leaf sheath. As the shoot and leaf elongated there was a separation except where held firmly together by this fungous stroma (Fig. 2, B). With many leaves and stems bound together in this manner the elongating culms also characterized by internodal dwarfing were deflected from their normal course and prevented mechanically from growing erect. Coincident with dwarfing and fasciation the inflorescences of diseased shoots were aborted or at least sterile, whereas the near-by healthy shoots bore normal florets. The mass of culms and leaves so dwarfed and distorted present an appearance strikingly similar to that recorded for the "Pokka Bong" disease of sugar-cane, which is due to a different fungus.

The record by Vizioli<sup>1</sup> and that by Freise<sup>2</sup> of *Myriogenospora aciculi*

<sup>1</sup> Vizioli, Jose. Estudo preliminar sobre un novo pyrenomyceto parasita da canna. Bol. Agr. São Paulo 27: 60–69. 1926.

<sup>2</sup> Freise, F. W. Cane diseases and plagues in Brazil. Facts about Sugar 25: 613–614. 1930.

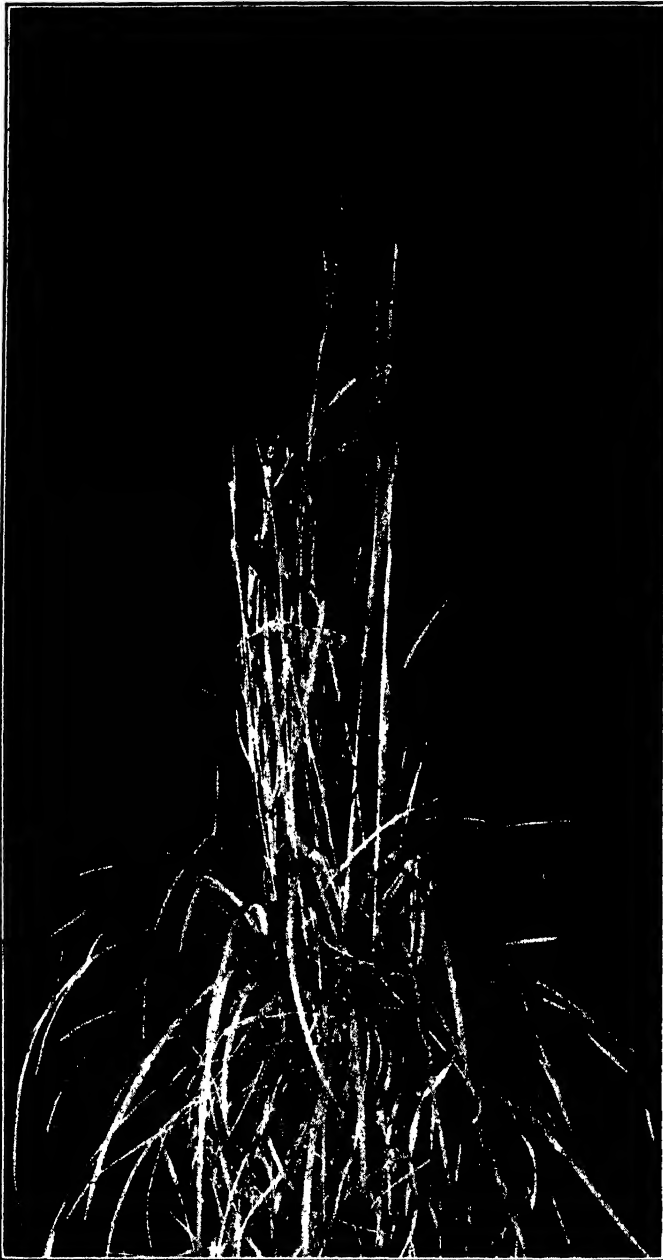


FIG. 1. Clump of *Andropogon scoparius* infected by *Myriogenospora paspali* and showing a fasciated and dwarfed condition. At right and rear are normal shoots which do not bear the fungus. Specimen growing in 6-inch pots in the greenhouse.  $\times \frac{1}{2}$ . Photographed by M. L. S. Foubert.





FIG. 2. Enlargement of parts of figure 1. A. Part of fasciated plant showing the attachment of leaf tips to near-by leaf blades and culms.  $\times 1.5$ . B. Folded leaf blade in region of tip adherent to black stroma of *Myriogenospora* along the midrib of the adaxial surface of another leaf. Note two rows of ostioles with extruding spore masses along the black stromatic surface.  $\times 5.3$ . Photographed by M. L. S. Foubert.

*sporae* Vizioli in Brazil note a similar attachment of sugar-cane leaves by the mechanical binding of the stroma but do not describe the fasciation observable in *Andropogon scoparius*. In so far as the writer has been enabled to observe grasses in the southern United States affected by *Myriogenospora*, the fasciation is most severe upon *Andropogon*, although the effect upon certain other grasses is pronounced. Freise's (*l.c.*) account of the *Myriogenospora* disease of sugar-cane in Brazil indicates local severity but of minor economic consequence. *Myriogenospora* is not known from the eastern hemisphere, but that the genus is widespread upon *Andropogon*, *Paspalum*, *Axonopus*, and *Panicum* in tropical and subtropical America east of the Cordilleras is attested by herbarium specimens. Such records, however, convey no hint of the severity or prevalence of the disease.

It was thought that the inoculum provided by the diseased clump of *Andropogon* might be used to indicate how the diseased condition is engendered in nature. Hence, during the autumn of 1931 various attempts were made to re-infect artificially the 5 clumps of healthy *Andropogon scoparius* that had previously shown the disease. Attempts were made also to infect an equal number of potted clumps of the same species transplanted from near Falls Church, Virginia, as well as 5 plants of *Paspalum dilatatum* grown from seed in the greenhouse. During the same season and the succeeding winter attempts were likewise made to infect sugar-cane, using for a medium P. O. J. No. 228,<sup>3</sup> which is the clone recorded by Vizioli (*l.c.*) as host for his *M. aciculisporae*. These inoculations were all carried on in the greenhouse under the same high temperature conditions (70–100° C.) which prompted germination of ascospores in water. In no case was there any success in securing infection. The methods used were as follows:

1. Spraying over all of a tap-water suspension of germinating ascospores, followed, except for the sugar-cane, by being kept for 48 hours in a darkened moist chamber.
2. Hypodermic injection of germinating ascospores in tap-water suspension into leaf spindles, nodes, and internodes of the various grasses noted.
3. Placing of bits of stromata bearing germinable ascospores between the leaf sheaths and culms.

The fungus concerned is here called *Myriogenospora Paspali* Atk., although there may properly be some question as to this identification, since most records of the fungus upon *Andropogon* refer it to *M. Bresadoleana* P. Henn., a species also originally described on *Paspalum*. The 3 recorded species of the genus *M. Paspali*, *M. Bresadoleana*, and *M. aciculisporae* Vizioli are not described as essentially different. The slight differences in the recorded ascus and spore dimensions for these species do not distinguish

<sup>3</sup> Thanks are due to Dr. E. W. Brandes and Dr. Julius Matz for the initial plants of P. O. J. No. 228.

them. Moreover, the actual dimensions of asci and spores in living material of this fungus on *Andropogon* might identify it with either of these 3 species. The identification of this fungus on *Andropogon* with Atkinson's species must, therefore, be tentative.

#### DISCUSSION AND CONCLUSIONS

The loss of the diseased condition coincident with the disappearance of the fungus, *Myriogenospora Paspali*, suggests a causal relationship and shows definitely an example of recovery of a grass from a partially systemic disease.

Since there is no chlorosis or early death of infected shoots, the dwarfing and fasciation, as well as sterility, of the grass accompanying the fungus are nevertheless of peculiar pathological significance.

The fasciation appears to be due chiefly to a mechanical binding by fungous tissue of adjacent leaves and culms during the growth of the shoot.

The artificial inoculations, while ineffective, suggest that with the particular strain used infection by *Myriogenospora* may not take place except through the seed at the time of seed setting or else at a period of susceptibility when seeds germinate. Neither of these two types of infection could, however, have taken place in Brazil with the sugar-cane clone P. O. J. No. 228, because of its Javanese origin and its apparent freedom, except in South America, from infection by the fungus. *Myriogenospora aciculisporeae* Vizioli would, therefore, appear, in the absence of reports elsewhere, to behave differently from the fungus on *Andropogon*. The fact that in the Southern United States *Myriogenospora* is not known on sugar-cane, although it is not uncommon on native grasses in the same region, suggests an immunity from available strains of the fungus in the clones grown there.

MYCOLOGY AND DISEASE SURVEY

BUREAU OF PLANT INDUSTRY

# NECTAR AND RAIN IN RELATION TO FIRE BLIGHT

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(Accepted for publication August 17, 1933)

Several workers during the past 15 years (2, 3, 5) have noted that the dissemination of the fire-blight organism, *Bacillus amylovorus*, is increased during wet weather, and some have adopted the view that splashing rain is the principal agent of dissemination. While it is a demonstrable fact that rain water may carry the organism from a holdover canker to blossoms on the same tree, there is no clear evidence that a wider dissemination is due to this agency.

The nectar of fruit-tree blossoms has not been critically studied in relation to fire blight, although it has long been accepted that the organism multiplies in the nectar and frequently penetrates through the nectary into the blossom.

In earlier work with the fire-blight disease, the writers shared the seemingly common impression that the nectar of fruit-tree blossoms rarely exceeds 20 per cent in concentration of total sugar. In the spring of 1932, George H. Vansell<sup>1</sup> called the attention of the writers to the work of Miss Beutler (1) in Germany and to his own unpublished work (6) in California, showing that the concentration of sugar in the nectar of fruit trees may vary from 2.3 per cent to 55 per cent or even higher. It was further shown that the variation in sugar concentration stands in close (inverse) relation to the atmospheric humidity and that the volume of nectar varies widely in direct relation with humidity.

The question at once arose whether the fire blight bacteria would be capable of multiplication in these more concentrated sugar solutions. Some tests were, therefore, made to determine the maximum concentration of the sugars dextrose, levulose, and sucrose,<sup>2</sup> that the organism will tolerate in culture solutions. Solutions were prepared by adding the desired quantity of sugar to the following nutrient solution adjusted to pH 6.9: magnesium sulphate, 2 gm.; dipotassium phosphate, 1 gm.; asparagin 3 gm.; distilled water 1000 gm.

Ten different cultures of the blight organism from as many sources were grown in each concentration of sugar. In dextrose solutions growth was optimum at 3 per cent, feeble at 14 per cent and very slight at 24 to 28 per cent. Several cultures made no growth at 16 and 18 per cent. In solutions of levulose, the highest concentration that permitted any growth was

<sup>1</sup> Associate Apiculturist, Bureau of Entomology, United States Department of Agriculture.

<sup>2</sup> The sugars that are found in nectars.

18 per cent and the optimum was near 10 per cent. In sucrose solutions growth was optimum at 10 per cent, but all cultures made feeble growth at 50 per cent and 2 cultures made very feeble growth at 58 per cent. It was found that the organism is able to survive for several weeks even at 60 per cent of sucrose but without perceptible growth and, upon recovery, is distinctly attenuated, requiring several passages through green pear fruits to regain its original virulence. It may be pointed out that the nutrient solution used in these tests, aside from excess of sugar, is highly favorable to the growth of the organism, while nectar is held to be low in all solutes except the sugars. In preliminary tests with pure extracted honey, which contains chiefly reducing sugars, a dilution of 1 to 8 in water was necessary before appreciable growth of the organism was obtained. In general, the concentration of sucrose, which inhibits the organism, is approximately double that of dextrose and levulose, a difference that is more or less parallel with the difference in the osmotic values of these sugars. However, the 2 reducing sugars seem to possess relatively somewhat stronger bacteriostatic power than is referable to simple osmotic relations.

It is apparent from these results that the maximum total sugar concentration tolerated by the organism will depend upon the proportion of the different sugars present in the nectar. Beutler presents some figures showing the relative amounts of saccharose and invert sugar for apple, crab-apple, cherry, and plum and states that the nectar of fruit-tree blossoms in general contains these sugars in a ratio of about 1:1. The writers have found no definite record in the literature for the sugar content in the nectar of pear blossoms.

Three samples of nectar from Bartlett pear blossoms collected at different dates were analyzed through the kindness of Mr. V. W. Smart of the Division of Plant Nutrition. In 2 of these no sucrose was detected and in the third only 11 per cent of the total sugar was measured as sucrose. Single samples of quince and apple nectar yielded 29 and 34 per cent sucrose, respectively. The method of Benedict and Osterberg as adapted by Thomas and Dutcher (4) was used in these analyses. Even disregarding the limited volume of nectar secreted in a dry atmosphere, it seems unlikely that the blight organism will increase appreciably in pear nectar after the concentration of sugars reaches 20 to 30 per cent. The comprehensive tests (by refractometer) of Vansell and a considerable number of determinations by the writers, indicate that higher concentrations are by no means infrequent in the comparatively dry air of the principal pear-growing areas of California. Vansell found pear nectar so low in volume at Davis and Placerville in March and April, 1932, as to preclude satisfactory sampling.

A few observations and tests were made at Berkeley in 1932 bearing on

the amount and concentration of nectar in pear, apple, quince, and cherry blossoms. These were examined from time to time during the early part of April but at no time was nectar found in abundance. Indeed, several attempts to obtain, under natural conditions, a sufficient quantity of nectar for refractometer determinations, met with indifferent success. However, when detached blossoms of apple, quince, and cherry were placed over night in an atmosphere of high humidity, nectar was produced in considerable volume, and the total sugar concentration under these conditions ranged from 6 to 11 per cent.

During the blossoming season of 1933, nectar was found in measurable quantities in pear blossoms at Davis and Berkeley. Both were on sunny days with comparatively dry atmosphere after early morning, and in both instances the total sugar concentration changed from 8 to 10 per cent at 9:00 a. m. to 20 to 22 per cent at noon after which samples were not obtainable by pipette. Samples of nectar taken from bees working in pear blossoms between 3 and 4 o'clock of the same day (at Davis only), contained 38 to 50 per cent of total sugar. On a drier day more nearly typical of that blossom period more than 100 blossoms were required at 6:30 a. m. to yield sufficient nectar for a refractometer reading, which, in this case, was equivalent to 18 per cent sugar. The rate of change from concentrated to dilute nectar has not been followed in the field. Detached pear blossoms, collected during a dry period and placed in an atmosphere nearly saturated with moisture, yielded nectar containing 5 per cent of sugar after 4½ hours, about 4 per cent after 7 hours, and 2½ per cent after 48 hours. In one instance the nectar was collected from 75 pear blossoms after these had been kept for 63 hours in a moist chamber. These averaged 1 cubic centimeter of nectar for each 25 blossoms and the concentration of sugar was less than 2 per cent.

Pear nectar has not been found in the orchard in sufficient volume to be studied as a culture medium. Nectar from plum sterilized by filtration and containing 3.2 per cent sugar, permitted vigorous growth of the fire-blight organism.

Apple blossoms were brought into the laboratory and atomized with a suspension of the fire-blight organism. These were then divided into 2 lots. One of these was dried for an hour in the draught from an electric fan, and the other was placed in an atmosphere of high humidity. The nectar in the latter soon increased to a large drop in each blossom with a total sugar concentration of about 5 per cent. After 2 days the filaments in contact with the nectar were discolored and in 5 days typical symptoms of blossom blight were apparent. The blossoms kept in dry laboratory air remained free from symptoms 7 days after the time of inoculation, when they were discarded.

In 2 similar experiments with pear blossoms, similar results were obtained. In a third test approximately half of the blossoms in the laboratory air became blighted, while all of those in the more humid atmosphere developed symptoms. The fan was not used to hasten drying in the experiments with pears.

It is probably significant that the principal blossom periods of pears in the Sacramento Valley in 1932 and 1933 were accompanied by prevailing dry winds and very few blossom infections. It also is of interest that in the epiphytotic season of 1930, precipitation was below normal at Sacramento for April, May, and June but atmospheric humidity was unusually high at certain periods.

The foregoing facts seem to the writers to offer a more reasonable explanation for the observed acceleration of fire blight during periods of high relative humidity than does the assumption that rain functions directly as a principal vector.

#### SUMMARY

The nectar of fruit-tree blossoms grown in a dry atmosphere is found to contain sugars in concentrations considerably greater than those that will permit growth of the fire-blight organism in culture solutions. It is proposed that the increase in volume and the reduction in concentration of nectar during humid weather bears an important relation to the incidence of fire blight.

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## PHYTOPATHOLOGICAL NOTES

*Physiologic Specialization in Puccinia glumarum in the United States.*— Since the discovery of *Puccinia glumarum* (Schmidt) Eriks. and Henn. in the United States by Dr. F. Kølpin Ravn, in 1915, physiologic specialization in this species has been of special interest. Aside from the work of Hungerford and Owens,<sup>1</sup> in which they state “that the rust which attacks *Hordeum murinum* may not be the variety commonly found on wheat,” there has been until recently little or no evidence of more than one physiologic form of *P. glumarum* in the United States.

On July 18, 1933, in the uniform stripe-rust nursery grown in the Flat-head Valley of Montana, the wheat variety Red Russian (C. I. 5409) was found to be susceptible to the form of *Puccinia glumarum* prevalent in that section. This same variety, however, always has been immune from the form commonly present at Moscow, Idaho.

At the time of the nursery reading a goodly amount of the rust was collected for further study under controlled conditions of light and temperature at Moscow. The culture was increased on Pannier barley until transferred to the various wheat-variety testers for a comparison with the Moscow form. On December 5, 1933, a number of wheat varieties used by Gassner and Straib<sup>2</sup> in their determination of physiologic forms of *Puccinia glumarum* in Europe, together with some American varieties of wheat and emmer, were inoculated with the Montana culture. After inoculation the plants were kept in a temperature-and-light controlled room at 52–55° F. and were given 12 hours of light, daily, supplied by a 500-watt electric light. They were exposed to the above conditions for a 30-day period and then were read for their reaction to the rust. Later, different plants of the same varieties were inoculated with the form of *P. glumarum* common to Moscow, Idaho, and were exposed to the above-mentioned environmental conditions. The results of these two tests are given in detail in the following table.

From the above table it is apparent that the varieties Red Russian and Chinese 166 differ decidedly from the other varieties in the reaction to the two collections of *Puccinia glumarum*, both being resistant to the Moscow collection and susceptible to the one from Montana.

Because of these differences and because the two collections were tested under identical environmental conditions, the writer believes there is suffi-

<sup>1</sup> Hungerford, Charles W., and C. E. Owens. Specialized varieties of *Puccinia glumarum*, and hosts for variety *tritici*. Jour. Agr. Res. 25: 363–402. 1923.

<sup>2</sup> Gassner, G., und W. Straib. Die Bestimmung der Biologischen Rassen der Weizen-gelbrostes (*Puccinia glumarum* f. sp. *tritici* (Schmidt) Erikss. u. Henn.). Arb. Biol. Reichsanst. Land- u. Forstw. 20: 141–163. 1932.



TABLE 1.—The reaction of a number of wheat varieties to two stripe-rust collections

Variety	Moscow, Idaho, collection			Flathead Valley, Mont., collection		
	No. of plants inoculated	No. plants showing infection	Responses <sup>a</sup>	No. of plants inoculated	No. plants showing infection	Response
Webster (C. I. 3780)	10	6	R	9	7	R
Red Russian (C. I. 5409)	9	0	A I	11	5	S
Triplet (C. I. 5408)	10	2	S	12	6	S
B. Winter Emmer (C. I. 2337)	11	10	S++	12	6	S++
Heines Kolben	14	10	S	12	7	R-
Vilmorin's Blé rouge d'Ecosse	11	0	A I	11	0	A I
Rouge Prolifique barbu	12	0	A I	9	0	A I
Chinese 166	13	1	R++	13	9	S++
Holzapfels Früh	11	0	A I	11	0	A I
Spaldings Prolific	10	0	A I	12	0	A I
Michigan Amber 29-1-1-1	14	1	S	12	7	S++
Strubes Dickkopf	10	0	A I	11	0	A I
Vilmorin 23	13	0	A I	9	0	A I
Carstens Dickkopf V	9	0	A I	11	0	A I

<sup>a</sup> A I = Apparently immune; R++ = extremely resistant; R = moderately resistant; R- = fairly resistant; S = moderately susceptible; S++ = completely susceptible.

cient evidence to warrant the conclusion that they represent two distinct physiologic forms, the Montana form being the one evidenced for the first time in the United States.

A more detailed report of these findings will follow.—WAYNE M. BEVER. Cooperative investigation between the Idaho Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

*Crown Gall of Hops.*<sup>1</sup>—Crown gall of hops has undoubtedly been present in Pacific Coast yards for many years. The association of *Phytomonas tumefaciens* (Smith and Town.) Committee S.A.B., with the disease has been definitely recognized since 1907. Smith, Brown, and Townsend made exhaustive preliminary studies of the organism isolated from galls on a number of plants, including hops. By means of artificial inoculations with the organism obtained from hop galls they reproduced the disease on almond, daisy, grape, hops, sugar-beet, and tomato plants. Their attempts to infect cotton, olive, and peonia were unsuccessful. The crown-gall organism from daisy galls was successfully transmitted to English Cluster and Custis Late varieties of hops. The organism from peach galls likewise was transferred to hops. These authors state, "The reports of hop growers on the Pacific Coast indicate that this disease may do considerable damage, particularly as the galls often reach a diameter of one's double fist. Some believe that an attack of two years' duration is sufficient to kill a plant. According to Dr. W. W. Stockberger of this Bureau, the disease occurs on hops not only in Washington State and Oregon, but also in the Sacramento valley in California. There I have seen acres of hops in which scarcely a hill could be found which did not show these tumors, some of them being larger than my fist."<sup>2</sup>

What appears to be the first official report to the Plant Disease Survey of the United States Department of Agriculture of the presence of the crown gall of hops on the Pacific Coast was made on September 1, 1929, when a 35-acre Polk county, Oregon, yard of the Late Clusters variety was found in which some 10 per cent of the plants were infected.<sup>3</sup> Subsequent meager reports of the incidence of the disease on the Pacific Coast disclose the fact that both the Early Clusters and Late Clusters varieties have been found infected in Benton, Linn, and Polk counties, Oregon, each year since 1929 to date. The so-called Bavarian hops also have been reported infected.

<sup>1</sup> Published as technical paper No. 221, with the approval of the Director of the Oregon Agricultural Experiment Station.

<sup>2</sup> Smith, E. F., Nellie A. Brown, and C. O. Townsend. Crown-gall of plants: its cause and remedy. U. S. Dept. Agr. Bur. Pl. Indus. Bul. 213. 1911.

<sup>3</sup> Report made by S. M. Zeller, Oregon Experiment Station, Corvallis.

A specimen of the disease on Late Clusters was taken in Sonoma county, California, in 1933.

Early in March, 1932, sexless plants of the Late Clusters variety were being rogued from a yard consisting of several hundred acres located in Polk county, Oregon. The roots of 400 of these rogued plants were examined, and 6.25 per cent were found to be infected with crown gall. Many of the galls were very extensive and involved entire crowns.

The vegetative characteristics of the host plant, which develops a perennial rootstock, is such that, in older plantings particularly, a high percentage of the plants might be infected without the fact becoming evident, or any appreciable damage being done, unless entire crowns become diseased and the development of all normal shoots from such diseased crowns were thereby prevented.

In hop nurseries, where underground stem cuttings are rooted and allowed to remain a year or more before being planted out in the main yard, crown gall is occasionally encountered in quantities sufficient to necessitate the destruction of a considerable amount of planting stock.

Otherwise, the disease, while doubtless quite general, cannot be considered of major economic importance.

The customary cultural practices to which hops are subjected afford ample opportunity for the spread of the disease. It undoubtedly is spread from yard to yard by planting infected or contaminated cuttings. Several specific cases are on record that would seem to confirm this statement. It is possible also that nematodes may be associated with the spread of the disease.

The writer recognizes 3 distinct types of gall: one of which involves the entire crown, and is comparatively uncommon. A second type of gall usually involves buds on the underground stems, and produces "hairy-root." The third and most common type of gall affects isolated portions of the underground plant parts or a portion of the crown at or near the ground level. In the field, the hairy-root type of gall disintegrates very readily, which also is true of the other 2 types when these growths occur on the crown near the soil surface and are subjected to severe winter weather.

What is presumed to be the causative organism has been isolated from both the second and third types of galls on Early Clusters, and Late Clusters varieties of hops. When the organism recovered from the last type of gall mentioned was used for artificial inoculations in the greenhouse, a similar type of gall was produced within 30 days on the crowns of both Fuggles and Late Clusters varieties.

It is of interest to note that A. J. Riker, to whom specimens of the second and third types of galls were sent, reported, after several trials, the inability of his associates to isolate a causative organism and suggested that



FIG. 1. Crown gall of hops. A. Exceptionally large gall on Early Clusters. B. Typical gall on Late Clusters. C. Hairy-root type of gall on Early Clusters.

the galls might be overgrowths brought about by some unfavorable physiological conditions rather than of bacterial origin. Later isolations made under Dr. Riker's supervision from the third type of gall resulted in the successful recovery of an organism with which tomato plants were artificially inoculated and similar galls produced.

Further and more detailed investigational work with this strain of the crown-gall organism from hops is being initiated.—G. R. HOERNER,<sup>4</sup> Oregon Experiment Station, Corvallis, Oregon.

*Methods for Determining Sex Differences in Sphacelotheca Sorghi.*—In genetic studies on *Sphacelotheca sorghi* (Link) Clinton, it is important to know the sexual capabilities of primary monosporidial lines, since paired lines of opposite sex are prerequisite to normal infection and production of chlamydospores in the host plant (5).

Heretofore, two methods have been used for determining sex in *Sphacelotheca sorghi*: 1. Rodenhiser (5) inoculated young sorghum plants hypodermically, in the greenhouse, with paired and nonpaired monosporidial lines and found that certain combinations of lines produced chlamydospores and others did not. This is a reliable test but requires considerable labor and time in waiting for results; 2. Rodenhiser obtained preliminary results indicating that when young sorghum plants were inoculated hypodermically the chlorosis of vegetative parts was indicative of the sexual compatibility of the lines used in inoculating. The writer has confirmed these results through extensive experiments. This second method has the advantage of making it possible to get results in a shorter time than by the first method.

In addition to the two methods already mentioned the writer has utilized sporidial fusions as a sex index in *Sphacelotheca sorghi*, as various workers have done with other smut fungi (1, 2, 3, 4, 6). Isenbeck, in unpublished data obtained at the University of Minnesota, stated that he observed rather scanty fusion between sporidia of *S. sorghi* in mixed cultures of monosporidial lines grown on nutrient agar. The writer observed numerous sporidial fusions in cultures of single chlamydospores and certain paired monosporidial lines on potato-dextrose and malt agar on cover slips over van Tieghem cells. The detection of fused sporidia is complicated by the presence of numerous non-fused cells and also by the types of fusion involved. Different stages of fusion were observed. Cultures of young, rapidly budding sporidia seem to be prerequisite to successful fusion, as very few or none were observed in mixtures of old stock cultures. This method is reliable and time-saving, but requires considerable experience.

A still simpler method is that used by Bauch on *Ustilago violacea*

<sup>4</sup> Agent, Division of Drug and Related Plants, United States Department of Agriculture.

(Pers.), *U. scorzonerae* (Alb. et Schw.), and *U. zae* (Beckm.) Unger (1, 2, and 3). The method follows: Four primary sporidial lines are isolated from a single chlamydospore. One line is grown in combination with the other 3 on slightly alkaline malt agar in a Petri dish. After incubation at the proper temperature, sex differences are indicated by the development of a fluffy white mycelium, the so-called *Suchfäden*. Simulating Bauch's technic, the writer obtained excellent results with *Sphacelotheca sorghi* (Fig. 1). Also, it was found that slightly alkaline potato-dextrose agar was

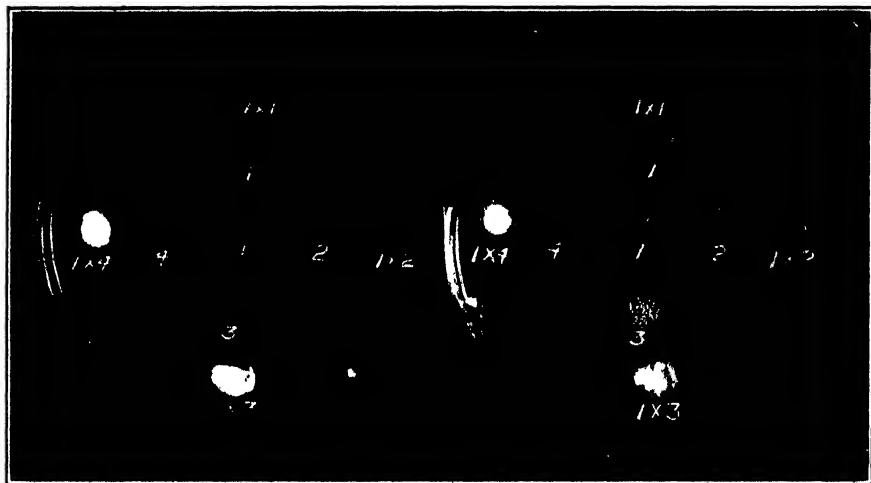


FIG. 1. Duplicate agar plates showing the results of the Bauch test on four primary monosporidial lines from a single promycelium of *Sphacelotheca sorghi*. Line 1 is the tester; material from the center colony is smeared on the colonies of lines 1, 2, 3, and 4 in the outer circle, while the colonies of the same lines in the inner circle are left as checks. It is evident that line 1 is of different sex than lines 3 and 4, while 2 is like 1. These results were confirmed by the sporidial-fusion method and by inoculating plants.

satisfactory, although the results were not so sharp as on Bauch's medium. In mixtures of those lines that reacted positively in the Bauch test there were fused sporidia as well as *Suchfäden*, while there were none in the checks. On the leaves of sorghum plants inoculated with lines that reacted positively on the Bauch medium, there were chlorosis and flecking, and, later, as the plants approached maturity, chlamydospores were produced. Lines with negative Bauch reaction also gave negative results in inoculated plants.

Holton (4) obtained inconsistent results with the Bauch test as applied to *Ustilago avenae* and *U. levis*. It is possible that he deviated too much from the procedure recommended by Bauch. In the writer's experience the method so far has been entirely reliable for *Sphacelotheca sorghi*.—LEON J. TYLER, University Farm, St. Paul, Minn.

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*A Procedure for Inducing the Production of the Sporangial and Swarm Stages in Certain Species of Phytophthora.*—Investigations with *Phytophthora* spp. have been greatly facilitated by the development of a method for inducing quickly and at a desired time the production of an abundance of zoospores. Mycelium from nutrient-agar slant cultures is transferred to weak prune-juice broth (60 gm. prune pulp in 1,000 cc. tap water) and the fungus incubated at, or 2° to 4° C. below, its optimum temperature for a period of 10 days to 2 weeks, when considerable growth will have been made. The mycelium is next transferred to a Petri dish having an amount of sterile tap water (about 8 cc. in a 95 mm. Petri dish), which will submerge approximately half of the mycelial transfer, permitting some hyphae to protrude above the water level. Zoosporangia form in large numbers; in the case of *Phytophthora citrophthora* they begin to make their appearance in 12 to 15 hours. Incubation in light appears to favor sporangium formation in this species.

Division of sporangial protoplasts into zoospores and emptying of sporangia are then induced by sudden exposure to 2 temperatures. The Petri dish is floated for 3 to 5 minutes in water having a temperature of 28° to 30° C. It is then transferred to water at 15° to 18° C. in which it is allowed to remain for an hour. Zoospores usually begin to emerge 15 to 20 minutes after transfer to the cooler water and are in greatest number after approximately 1 hour. Zoospores thus produced and kept at a temperature of 18° to 22° C. will retain their motility for 45 to 90 minutes. The method has been very useful in our laboratory for experiments in the prevention of infection of fruits by various disinfectants and fungicides and for testing and observing directly the effect of such substances on the motility and germination of the zoospores.

The idea of utilizing a sudden drop in temperature to induce swarming was suggested to us by our experiences with *Phytophthora* spp. in the laboratory and by the observations of H. J. Toxopeus reported by correspon-

dence from Poenten, Java, where he noted that the gummosis organism produced an abundance of zoospores during the monsoon rains that suddenly lowered the temperature.

The foregoing procedure has been successful with *Phytophthora citrophthora*, *P. parasitica* (*terrestris*) and *P. cactorum*. A discussion of the various factors involved in the method will be reported later. H. S. Fawcett and L. J. Klotz, Citrus Experiment Station, Riverside, California.

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*A Correction:* The Editor wishes to call attention to the fact that through an error in printing the contribution by L. O. Kunkel, entitled "Studies on acquired immunity with tobacco and aucuba mosaics" (*Phytopath.* **24**: 437-466. 1934), figures 2 and 7 occupy interchanged positions and each is accompanied by the legend of the other.



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## IDENTIFICATION OF CELERY VIRUS 1, THE CAUSE OF SOUTHERN CELERY MOSAIC

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(Accepted for publication September 16, 1933)

It is with pleasure that the writer thanks the University of Wisconsin, Madison, Wisconsin, the city of Sanford, Florida, and the Boyce Thompson Institute for Plant Research, Yonkers, New York, for furnishing laboratory and greenhouse facilities without which many points reported in this paper could not easily have been discovered.

### INTRODUCTION

The fact that diseases occurred that were caused not only by fungi and bacteria but also by viruses was first demonstrated before the year 1890. Since that time a large number of related maladies with their host ranges have been reported on both animals and plants. With phytopathological literature so filled as it is with data relative to these virus troubles, it becomes increasingly evident that mere report of a plant virus, even accompanied with an account of symptoms and its importance on the host, is not sufficient to place it in the category of a new and separate disease.

For over ten years a mosaic disease of celery has caused increasingly severe losses in Florida trucking areas, and has also been reported (5) from California, Wisconsin, Ohio, and New York, though it apparently has caused little damage in these latter States. The disease was reported from Florida in 1924 by Foster and Weber (7), but no experimental work had been conducted at that time. In 1929 a study was made of the disease by Doolittle (3) and it was continued in 1930 by Doolittle and Wellman (5). The last two authors have shown that it is a true mosaic transmitted both by juice inoculation and by the melon aphid, *Aphis gossypii* Glov., and was found to occur on a monocotyledonous weed, *Commelina nudiflora* L. Wellman continued work on this problem in the fall of 1930 and by 1932 had found (23) that eradication of this monocotyledonous weed host in the

<sup>1</sup> The writer thanks Dr. F. O. Holmes who gave him time, enthusiastic interest, and the benefit of broad experience on virus studies while at the Boyce Thompson Institute. Likewise, acknowledgment is due Dr. S. P. Doolittle for his interest in the problem and also Dr. J. Johnson for much constructive criticism.

vicinity of the fields was effective in eliminating in large measure losses due to the mosaic in celery fields.



FIG. 1. Southern celery-mosaic symptoms on Golden Self Blanching celery and on the weed host, *Commelina nudiflora*. A. Mosaic-diseased celery plant at harvest time, cut off at the ground line, and stripped as for market. Such a plant apparently is only half as tall as a healthy individual, the petioles, instead of being straight, turgid, and white, are shrivelled and discolored, and there is a curvature of both petioles and leaf rachises. The leaflets are brilliantly mottled, somewhat savoyed, and slightly twisted, but they are not upright in growth habit nor do filiformities occur as is the case in plants infected with common cucumber mosaic virus. (Compare with Fig. 5, B.) B. On *Commelina* the southern celery-mosaic virus causes an irregularly watered type of mottling pattern of an indistinctly marbled or moiré-like appearance. It is only rarely that such leaf malformations occur on *Commelina* as are shown on the left branch in this figure.

The southern celery mosaic is characterized by leaf mottling and sunken discolored streaks on leaf stalks, but no filiformities or "shoe string" effects are produced on leaflets (Fig. 1, A). As will be pointed out later in this paper, these leaflet malformations are common symptoms produced either by Doolittle's (2) cucumber-mosaic virus or the tobacco ring-spot virus described by Wingard (24). These leaf malformations are always spoken of in descriptions of symptoms on mosaic-infected celery plants by Poole (19), Elmer (6), and Harvey (9). Johnson and Grant (16), using *Cucumber virus 1*, Doolittle, have proved that the common cucumber mosaic virus is readily transmitted to celery. From the relative abundance of the common

cucumber virus and the symptom expressions on diseased celery described in their publications, it is believed that Poole, Elmer, and Harvey probably were working with *Cucumber virus 1*.

Preliminary cross-inoculation studies by Doolittle and Wellman (5) with the southern celery-mosaic virus showed that it was transmissible to celery, cucumber, tomato, tobacco, and *Physalis pubescens*, and that it was successfully obtained from naturally infected celery, *Commelina nudiflora*, and *Physalis lagascae*. The symptoms on some of those hosts were somewhat similar to those caused by common cucumber-mosaic virus, and in some respects of other known virus diseases, notably, tobacco ring-spot. In view of these facts and the marked economic importance of the disease, it was evident that detailed studies of properties and identity of the causal virus were essential to the development of adequate control measures. In 1932 Wellman (23) had carried studies far enough to state the belief that the southern celery mosaic was probably caused by a virus distinct from the one causing common cucumber mosaic. As a result of these and later studies herewith reported, it is clear that the virus causing southern celery mosaic is specifically different from any heretofore described.

#### MATERIALS AND METHODS

Eight strains of viruses were used in these experiments for identification of the southern celery-mosaic virus and were from known sources. These have been listed in tabular form and are given in table 1. All viruses were acquired from living diseased plants obtained from the workers who so kindly furnished them, and, after being transferred to healthy greenhouse plants, they were checked as to symptoms produced on hosts and characteristic properties before being used as comparative material.

The original sources of southern celery-mosaic virus used in this work were a mosaic-infected celery plant and plants of the weed (*Commelina nudiflora*) obtained in an area of 10 square feet along the edge of a naturally diseased field in the Sanford, Florida, trucking district. Investigations soon proved that the weeds and cultivated plants were infected with the same virus. It has since been retained by continuous reinoculation methods. It has been carried in growing tobacco, cucumber, celery, and *Commelina* plants, and has been found to remain unchanged in its characteristics in any of these hosts through three years of such cultivation.

All of the earlier juice inoculation work reported by Doolittle and Wellman (5) was done by means of needle inoculation methods as described by Johnson (15), Doolittle (2), and many others. Using this technique it was possible to obtain a fair degree of transmission. However, in many cases such as dilution and thermal death-point studies, this technique was not satisfactory because erratic results were obtained, which were ex-

TABLE 1.—*Viruses used in comparative studies and identification of the southern celery-mosaic virus*

Virus	Name of disease	Authority	Source of virus	Date secured
Ring-spot	Tobacco ring-spot	Wingard (24) <sup>a</sup>	Wingard and Henderson (on tobacco) <sup>b</sup>	August, 1931
Tobacco mottle	Latent or "healthy" potato	Johnson (15)	Schulz (on Green Mountain potato) <sup>b</sup>	July, 1932
Cucumber virus 1	Common cucumber mosaic	Doolittle (2)	Doolittle (on cucumber)	October, 1930
" " "	" " "	" "	Johnson, J. (on tobacco) <sup>b</sup>	April, 1931
Tobacco virus 1	Ordinary tobacco mosaic	Allard (1)	Doolittle (on tobacco) <sup>b</sup>	January, 1931
" " "	" " "	Johnson, J. (15)	Holmes (on tobacco)	June, 1932
Celery virus 1 <sup>c</sup>	Southern celery mosaic	Loc. cit.	Edge of Florida celery field (on celery) <sup>b</sup>	November, 1930
" " "	" " "	" "	Edge of Florida celery field (on <i>Com- melina nudiflora</i> )	October, 1930

<sup>a</sup> See "Literature Cited."<sup>b</sup> The strain used in principal comparative studies.<sup>c</sup> Name given at close of studies.

plainable only on the basis of grossness of method. Attention was therefore turned to finding a more delicate measure of infectivity of variously treated inocula. Comparative experiments with tobacco, cucumber, and celery plants showed that Holmes' wiping method of inoculation (12) was superior to methods of injection, needle prick inoculations, and leaf mutilations.

Larger percentages of infection were commonly obtained by this method and in many cases of extreme virus dilution, where no infection was obtainable by other means, a high percentage of plants succumbed to the virus when the inoculum was applied by the wiping method. A search was then instituted to discover a host that, when inoculated, would produce primary lesions, such as were obtained on beans by Price (20) and on *Nicotiana glutinosa* by Holmes (12), when treated with the ordinary tobacco virus. Inoculations were made on a large number of hosts susceptible to the southern celery-mosaic virus and it was found that well expanded cotyledons of cucumber (White Spine variety) developed small, typical virus lesions that could be counted. The results obtained were consistent and comparatively satisfactory.

The wiping method of inoculation was used on cotyledons of cucumber seedlings developed from 10 to 15 days after planting seeds. The cotyledons were green and fully expanded, and the true leaves were small and in the form of a loosely folded rapidly expanding bud. Care was taken to have the plants in a fully turgid condition at the time of inoculation. A small square of cheesecloth was wetted with the inoculum and held in the right hand, while the fingers of the left supported the cotyledon to be wiped. This operation consisted of 10 gentle but firm strokes on each cotyledon from the stem end to the tip in such a way as to cover the cotyledon surface at each stroke. Cotyledons are very tender, brittle, and easily broken so some care must be exercised to avoid undue injury to the rubbed surfaces. After inoculation, about 3 to 5 minutes were allowed to elapse and the cotyledons were then washed with a jet of water to remove excessive materials, which if allowed to remain, may produce a general necrosis of the cotyledons. This latter washing has been studied and seems to have no depressing effect on the numbers of lesions produced. After each series of plants was inoculated the hands were always washed with soap and water and dried.

Development of primary lesions on cucumber cotyledons (Fig. 2) ordinarily takes 3 to 7 days, depending upon conditions. Counts were made by observing the underside of cotyledons, since an occasional lesion could be found on this surface, that was practically invisible from the upper side. Although the inoculum is applied to the upper side of the cotyledons, the undersurfaces are often the most distinctly marked and have shallow, dark

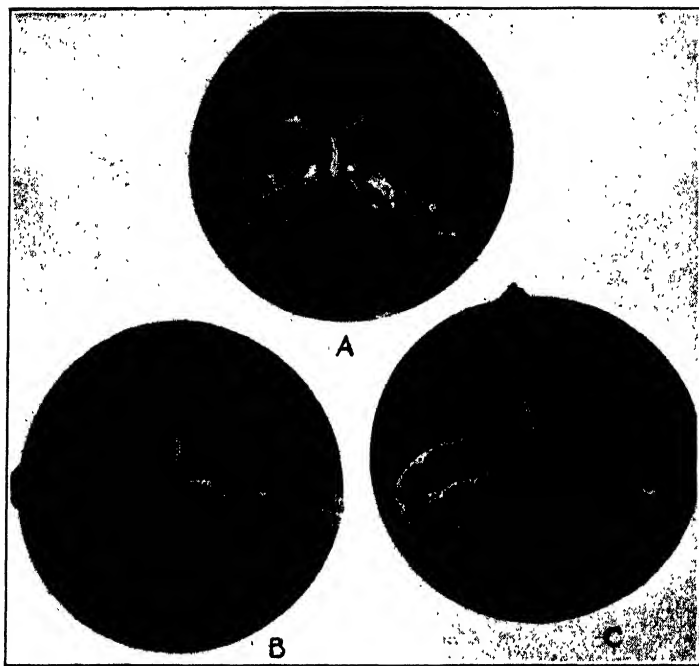


FIG. 2. Appearance of primary lesions of southern celery-mosaic virus on cucumber (White Spine) seedlings. A. Seedling infected by inoculating upper surface of cotyledons with juice from a celery plant diseased with the southern celery-mosaic virus. Note that 16 primary lesions are visible when these 2 cotyledons are observed from the upper surface. When these same cotyledons were turned over they were found to have a total of 19 primary lesions. Note also that the central bud seems to be tightly folded, the first true leaf has bent down and is dwarfed, and the infected cotyledons are drooping. B. Seedling whose cotyledons were inoculated with juice from a mosaic-free celery plant. Note that cotyledons appear turgid, are held in normal horizontal position, the first true leaf is expanding, and the bud containing the succeeding leaves is unfolding. C. Untouched, control seedling.

green, circular pits at the point of development of each primary lesion. Counts were made as promptly as possible after the lesions appeared, since they soon develop a necrotic condition that spreads, extends into the surrounding portions of the cotyledon, and renders the obtaining of numerical data uncertain.

It was found that the type of lesions and systemic symptoms developed by this virus (Fig. 1, A) is characteristic and may be relied upon for a criterion as to whether or not the celery virus is present. The virus multiplies in the cotyledons, and inoculations with juice from cucumber seedlings thus affected have regularly produced typical southern celery mosaic on celery.

Most of the work on properties has been done under all manner of seasonal conditions and in greenhouses located in widely separate States, Florida, Wisconsin, New York, and Virginia. Wherever the work has been done and under all conditions encountered the results have been characteristic.

#### EXPERIMENTAL RESULTS

##### PROPERTY STUDIES<sup>2</sup>

*Filterability of the Celery Virus.* Filtration experiments were not extensive. The fine grade "W" Berkefeld filters used were prepared, being washed and soaked in several changes of sterile distilled water. The filter candles were then set up in suction flasks, with a glass test tube so arranged in each flask that the filtrate would drip into the tube. These set-ups were then steam-sterilized, allowed to cool in a dust-free atmosphere, and finally attached with an intervening trap flask to a water pump which used regular city-water pressure to produce vacuum.

Eleven filtration tests were made using infective juice from plants of several ages. From 75 to 100 cc. of juice was obtained for each experiment and after being expressed from the diseased plants was strained through a double thickness of cheesecloth and poured over the filter candle. Filtration was fairly slow, but, within an hour after starting the suction pump, sufficient filtrate was obtained under aseptic conditions for tests to be made as to presence of both bacteria and virus. Regular bacteriological technique was used to test filtrates and unfiltered extracts for the presence of bacteria. Loopfuls of the liquids were plated out on neutral potato-dextrose and nutrient agars, and were also used to inoculate tubes of nutrient broth, which were incubated at between 24° and 28° C. for 14 days. In all, over 200 tubes and plates of media were inoculated in these tests. After these transfers, the remaining liquid was used to study the presence of virus. Small squares of sterilized cheesecloth were moistened with the remaining portions of filtrate and unfiltered extract and tobacco seedlings inoculated by the wiping method. From 5 to 15 plants were inoculated with the filtered and unfiltered juices and observed for 3 weeks before being discarded.

In the accompanying table 2 results from 7 representative experiments are listed. It will be seen that in these experiments all samples of plant juices before filtration contained bacteria and none after filtration. In 2 experiments (Table 2, Expts. 3 and 7) juice from diseased cucumber and tobacco plants, inoculated while quite large, was used. The cucumber

<sup>2</sup> The writer acknowledges the help of Dr. T. J. Grant, acting as collaborator in the U. S. Department of Agriculture, at Madison, Wisconsin, who independently corroborated many of his findings with regard to the properties of the southern celery-mosaic virus. However, the author takes full responsibility for all data reported and any errors are of his own derivation.

TABLE 2.—*Results of studies on filtration of southern celery-mosaic virus through fine ("W") Berkefeld filters, using Broadleaf tobacco seedlings as test plants for presence of virus, and potato and nutrient agars and nutrient broth to test for presence of bacteria*

Expt. no.	Inoculum		Presence of bacteria	No. mosaic plants
	Source	Treatment		
1	Old tobacco, mild symptom ...	Unfiltered	Present	15 <sup>a</sup>
	“ “ .....	Filtered	Absent	0
2	“ “ .....	Unfiltered	Present	14
	“ “ .....	Filtered	Absent	0
3	Old cucumber, <sup>b</sup> severe symptom .	Unfiltered	Present	15
	“ “ .....	Filtered	Absent	3
4	Young cucumber, “ .....	Unfiltered	Present	15
	“ “ .....	Filtered	Absent	15
5	Seedling cucumber, “ .....	Unfiltered	Present	15
	“ “ .....	Filtered	Absent	15
6	“ “ .....	Unfiltered	Present	15
	“ “ .....	Filtered	Absent	15
7	Old tobacco, <sup>c</sup> “ .....	Unfiltered	Present	13
	“ “ .....	Filtered	Absent	2

<sup>a</sup> Numbers in this column represent total plants diseased from 15 inoculated by the wiping method.

<sup>b</sup> These plants were inoculated when past seedling stage and after the fifth true leaf was expanded.

<sup>c</sup> These plants were inoculated after the seedling stage and when 10 leaves were well formed.

juice (Table 2, Expt. 3), before filtration, produced 100 per cent diseased plants, or 15 diseased of 15 inoculated. After filtration, infection was reduced to 20 per cent, or 3 diseased of 15 inoculated. The tobacco juice (Table 2, Expt. 7), before filtration, produced 87 per cent infection, or 13 diseased of 15 inoculated, and after filtering infection was reduced to 13 per cent, or 2 diseased of 15 inoculated. Three experiments (Table 2, Expts. 4, 5, and 6) were performed using juice from cucumber seedlings 6 days after inoculation, which was the day serious systemic infection first appeared. Non-filtered plant juices were highly infective, giving 100 per cent of severe disease in all cases. After filtration these juices were still infective producing 100 per cent disease in the 3 experiments, but systemic symptoms on test tobacco seedlings were delayed in appearance from 1 to 3 days, which indicated a lessened concentration of virus in the filtered inocula. In 2 experiments, listed as numbers 1 and 2 in table 2, the virus was obtained from tobacco plants that had been systemically diseased for over 30 days. Mosaic symptoms in these plants were mild. These juices were good inocula before filtration, but after filtering they were apparently



free from the virus. These studies were carried further in subsequent experiments not represented in the accompanying table. In 3 cases mosaic-infected celery plants were used as sources of inoculum in which filterability of the virus in celery juice also was proved. Of 17 tobacco plants inoculated with bacteria-free filtrate from diseased celery plants, 5 developed symptoms typical of the southern celery-mosaic virus. The unfiltered juice produced 100 per cent disease in 17 control plants inoculated.

It would appear in filtration studies of this virus that infective filtrates are more readily obtained from juices containing a high concentration of the virus (Table 2, Expts. 4, 5, and 6) than from those juices where the virus concentration is low. In later studies expressed juice from severely diseased cucumber seedlings, such as those used in the experiments just cited, produced a total of over 150 primary lesions on 10 cucumber cotyledons. Juice from old tobacco plants, such as that used in experiments 3 and 7, listed in table 2, produced about 30 to 60 lesions.

*Thermal Death Point.* A common factor considered in determining differences in virus characteristics is that of the temperature at which the infective juice loses its disease-producing qualities. Because of its convenience as a term and its analogy in bacteriological studies, this induced inactivation of the virus by heating is called "death" in this discussion. The technique used by Grant and described in a paper by Johnson and Grant (16) was followed in these studies. The experiments were repeated over a period of several months. The work covered spring, summer, and fall growing conditions; and celery, cucumber, and tobacco plants were used both as test hosts and as sources of inoculum. With minor and expected deviations the results from all tests were markedly alike. (Table 3.)

It was found that occasional samples of viruliferous juices remained infective after treatment at 75° C. for 10 minutes, but in all cases the virus was dead in samples treated at 80°. It is probable that the ultimate point of death is no more important than the point at which first evidences of reduced infection result. The lower ranges of reduced infection with the virus apparently start a little below 50°. The lowest temperature at which a large reduction of virus infection results is below 65°. Freezing the virus seems to have little or no deleterious effect on its infective properties.

*Tolerance to Dilution.* In studying the dilutions at which juices of plants affected with southern celery mosaic would still remain infective, it was found that considerable variation existed dependent, apparently, upon type of host, succulence of plant, and vigor of disease development. Young diseased plants of cucumber, tobacco, celery, and *Commelina nudiflora* were the hosts from which fresh viruliferous juices were obtained for use in these studies. Dilution was with sterile water and the dilution made was used immediately as material for inoculation.

TABLE 3.—Data from experiments to show the effect of heating viruliferous plant juices for 10 minutes at different temperatures on disease production by the southern celery-mosaic virus

Source of inoculum	No. tobacco plants infected out of 5 inoculated								
	Temperature treatments (° C.)								
	20-24	45	50	55	60	65	70	75	80
Tobacco (Broad leaf) .....	5	5	5	5	4	0	2	0	—
“ “ .....	5	5	5	5	0	0	0	0	—
“ “ .....	5	—	5	—	5	—	1	0	—
Tobacco (Turkish) .....	5	—	—	4	—	2	—	1	0
“ “ .....	5	—	—	5	—	0	—	1	0
Cucumber (White Spine) .....	5	5	5	3	2	0	0	0	—
“ “ .....	5	5	5	3	1	0	0	1	—
Cucumber (Early Fortune) .....	5	—	—	5	—	2	—	1	0
“ “ .....	5	—	—	2	—	1	—	0	0
Celery (Golden Self Blanching) .....	5	5	4	4	0	0	0	0	—
“ “ .....	5	5	2	4	1	0	0	0	—
“ “ .....	5	—	2	—	1	0	0	0	—
“ “ .....	5	4	1	2	0	1	0	0	0
Celery (Special) .....	5	5	3	—	1	—	0	—	0
“ “ .....	5	5	1	—	1	—	0	—	0

Juice from young diseased cucumber seedlings when diluted 1:10,000 was infective, a total of 3 to 5 lesions being produced on a series of 10 inoculated cucumber cotyledons. Only an occasional lesion was produced on series of 10 cotyledons inoculated with juice diluted 1:100,000 and some series were entirely free from infection. Viruliferous extract from young tobacco plants was apparently less infective but one or two lesions occurred when 10 cucumber cotyledons were inoculated with juice diluted 1:10,000. Juice from diseased celery seedlings selected at random was erratic in behavior. The highest dilution of this material that gave infection was one of 1:1,000. The sap of *Commelina nudiflora* is viscous and the problem of diluting the juice of this host is being studied further. In the preliminary experiments made it was found that when viruliferous *Commelina* juice is diluted 1:500 or more no infection could be obtained when it was used as inoculum.

It is believed these experiments point out that data from dilution studies are complicated and are not good criteria to determine a virus characteristic, except, for instance, as they may indicate the freedom with which the virus has multiplied within a given host plant grown under given conditions.

*Tolerance to Aging in Vitro.* It is questionable just what factors may be involved in causing death of the virus when it is stored *in vitro* in unsterilized

and unfiltered from diseased plants. Though the data differ somewhat in separate experiments under a wide range of conditions it is apparent that the life of the virus of southern celery mosaic is fairly short when stored in plant juices. Juice from diseased cucumber seedlings, allowed to stand only 4 hours at a warm temperature ( $28^{\circ}$  C.), shows a considerable reduction in the infective properties of the southern celery-mosaic virus. On the other hand, it is interesting that viruliferous juice from cucumber seedlings may be kept at least 2 days at  $28^{\circ}$  and still retain some of its infectiveness. When kept at ordinary room temperature ( $18^{\circ}$  to  $20^{\circ}$  C.) the juice remains infective from 6 to 8 days. When the juice is stored at  $3^{\circ}$  C. it may remain infective for 2 weeks and possibly longer, though at the close of that time considerable reduction of infectivity of the virus apparently has occurred. If the viruliferous juice is frozen immediately after being expressed it retains its active virulence apparently unhampered over a period of at least 2 weeks. It has not been kept longer than that.

*Influence of Desiccation on the Virus.* The southern celery-mosaic virus is not destroyed immediately upon rapid drying. Tobacco seedlings were used as test plants in these studies. Squares of cheesecloth were dipped in infective juices from tobacco and cucumber plants and then air dried in a room at  $26^{\circ}$  C. Under these conditions the juices produced the disease after 72 hours drying. After 120 hours no infection could be obtained. However, infection was seriously reduced, even after the juices had dried 12 hours, and at the close of 24 hours of drying there was a 75 per cent reduction in diseased plants resulting from inoculation. After 72 hours of drying the virus infected only occasional plants (1 diseased in 15, 2 in 15, and 1 in 30).

Upon securing the above results the possibility of carrying the virus over in dried plant material was further studied. It appears that diseased leaf lamina of celery, cucumber, and tobacco lose the virus they contained when succulent when they become dry enough to crumble into powder. Numerous tests were made of such leaves from the 3 hosts, ranging in period after drying of 1 day to 5 months. No infection ever was obtained from them. Curiously enough, however, cucumber cotyledons that had numerous local lesions retained their infectious properties 2 days after being dry enough to crumble.

*Effect of Chemicals on the Virus.* It has not been possible to make exhaustive study of the effect of chemicals on the southern celery-mosaic virus. If properly carried out such a study would be long and involved, but in this case, only a few easily obtained chemically pure ("C.P.") laboratory chemicals have been used.

In all cases severely diseased cucumber seedlings, pulled 6 days after cotyledonary inoculation, have been used for the source of inoculum. After the stems, leaves, and cotyledons had been cut up and macerated, the pulp

was wrapped in a double thickness of cheesecloth and the juice pressed out. This was then diluted with an equal quantity of sterile water. Chemical solutions were all prepared as double-strength solutions. Equal quantities of the previously prepared virus solution were combined with an equal quantity of double-strength chemical solution. Take as an example of the method used in the mixing technique in these chemical treatment studies the 2.5 per cent sodium chloride treatment of the virus. Five cc. of freshly expressed viruliferous juice were mixed with 5 cc. of sterile water. Ten cc. of 5 per cent sodium chloride solution were added to this solution, shaken, and the mixture regarded as a 2.5 per cent solution of sodium chloride. Solutions were all prepared in this manner and then allowed to stand for 15 minutes. Small squares of cheesecloth were then wetted with the mixture and used to wipe over cucumber cotyledons, and in some cases the leaves of tobacco seedlings also were inoculated. After inoculation, the plants were allowed to stand 3 minutes and then washed with a stream of water (*cf.* Holmes (12)) to avoid killing the plants by chemical action. In some cases a large series of plants were inoculated and half of them left without washing. In some instances, *e.g.*, 5.0 and 2.5 per cent sodium chloride solutions, the plants were killed when the cotyledons and leaves were not washed. When washed, the test plants lived and allowed the virus remaining after chemical treatment to produce primary lesions.

The data from representative chemical-treatment tests are presented in table 4. Duplicate series have given comparable results when either cu-

TABLE 4.—*The effect of certain chemicals on the southern celery-mosaic virus*

Chemical	Strength of chemical (per cent)	Total no. of virus lesions on 10 cucumber cotyledons
Ethyl alcohol (absolute)	50.00	28
Hydrochloric acid (c.p.)	0.05	14
None		58
Nitric acid (c.p.)	1.00	0
Calcium hydrate (c.p. saturated solution)	50.00	0
Sodium chloride (c.p.)	10.00	0
“ “	5.00	2
“ “	2.50	19
None		56

cumber or tobacco seedlings were used. Consequently, results from such experiments have not been included in this table. It will be seen (Table 4) that, while 50 per cent ethyl alcohol was not completely harmless to the virus, it sufficed to cut down the resultant lesions to only half that obtained when the juice was not treated with any chemical. Solutions of 1 per cent

nitric acid, a 50 per cent saturated hydrated lime solution, and 10 per cent sodium chloride all acted as perfect inhibitants of virus infection when the otherwise infective juice was treated for 15 minutes. Treatments with weaker strengths of sodium chloride solution were made. A 5 per cent solution was apparently close to the critical point at which the virus would no longer be infective, since only 1 to 5 lesions developed; whereas untreated juices produced an average of about 60 lesions. A 2.5 per cent sodium chloride solution was less effective as an inhibitant than the 5 per cent mixture. It is interesting to note that a .05 per cent hydrochloric acid solution produced about the same effect on the celery virus that the 2.5 per cent sodium chloride solution did.

### Studies on Cell-Inclusion Bodies

A search for any notable differential cytological phenomena characteristic of the celery-virus disease has been carried on by the writer. The specificity of forms of "cell-inclusion bodies" may at times be questioned, but Kunkel (18) has shown that they are characteristic of a certain corn mosaic; Rawlins and Johnson (22) and Hoggan (11) show that they are commonly present in tobacco mosaic-infected solanaceous host tissues, while certain other viruses on the same hosts do not produce these curious bodies. Lately, Grant (8) has found that the ordinary tobacco virus when attacking various nonsolanaceous hosts is characterized by typical cell-inclusion bodies in the tissues.

In the present study the writer stained with Heidenhain's iron-alum haematoxylin severely affected chlorotic leaf tissues of plants affected with southern celery mosaic. However, no specifically differentiating cytological phenomena were found, and no cell-inclusion bodies of any sort appeared. The diseased plants were grown under conditions that were ideal for development of cell-inclusion bodies in ordinary tobacco-virus-infected plants, since the leaf tissues of tobacco plants, infected with ordinary tobacco mosaic, growing near-by, were found to contain such bodies. The following plants infected with the southern celery-mosaic virus were used in this study: celery, *Apium graveolens* L., cucumber, *Cucumis sativus* L., Broadleaf tobacco and Turkish tobacco, *Nicotiana tabacum* L., zinnia, *Zinnia elegans* Jacq., French marigold, *Tagetes patula* L., red garden beet, *Beta vulgaris* L., California bluebell, *Phacelia whittlavia* Gray, and Flora's paint brush, *Emilia sagittata* DC.

The affected portions of leaf tissues were considerably reduced in thickness, which was especially notable in the much shortened palisade cells and more compact spongy parenchyma. The numbers of chloroplasts appeared to be about the same as in healthy cells but they were very small and suppressed in development. Also the vascular strands were smaller in diame-

ter than comparable healthy tissues. But neither the so-called "x-bodies," "striate material," nor any other cell-inclusion peculiarity was observed in the tissues of any species of plants infected with southern celery-mosaic virus.

### Studies on Rate of Virus Spread

Under the summer growing conditions in the greenhouse at Arlington, Virginia, systemic infection by southern celery-mosaic and common cucumber-mosaic viruses in tobacco, tomato, and cucumber plants first appeared in those inoculated with the celery virus. The difference in time of symptom appearance was generally from 1 to 3 days but often 4. Sometimes this difference was not so marked in cucumber plants, especially young seedlings, but careful scrutiny seemed always to decide the rate of appearance of systemic infection slightly in favor of the celery virus.

During a few weeks' stay at the Boyce Thompson Institute, a study was made with F. O. Holmes to see if infection with the southern celery-mosaic virus might produce an iodine staining pattern similar to that obtained by him (13) with ordinary tobacco mosaic and to see if this method might lend itself to other investigations of the southern celery mosaic. Although exacting in time consumed, this simple technique gives an especially valuable picture of the mode of virus spread. It was found at the outset that starch-retention patterns were produced in Turkish tobacco leaves when inoculated with the virus of both southern celery and common cucumber mosaic, incubated and stained according to Holmes's directions. Comparable series were prepared in which starch-retention patterns of common cucumber-mosaic virus, ordinary tobacco-mosaic virus and southern celery-mosaic virus on Turkish tobacco and *Nicotiana glutinosa* were studied. The treated and stained leaves were pressed and preserved and used in comparative measurements and observations. Data from these experiments are presented in table 5.

Within 24 hours, after inoculating Turkish tobacco leaves, celery mosaic caused small but distinct iodine-staining lesions that averaged 1.4 mm. in diameter. No lesions were observable in leaves inoculated either with common cucumber or ordinary tobacco mosaic. Thirty hours later the cucumber-virus lesions were just appearing and were about .3 mm. in diameter in affected leaves. Still there was no observable symptom of tobacco-virus infection. At the close of the 48-hour period after inoculation, the tobacco virus had caused lesions that were about half the size of celery-virus lesions noted 24 hours previously. Once tissue infection was established, the 3 viruses appeared to progress at considerably different rates.

In all 3 cases a certain amount of increase in area of affected tissue occurred about the original points of primary infection. The diameter of the

TABLE 5.—*A comparison of the time of production and progress of starch-retention patterns produced by the viruses of ordinary tobacco, common cucumber, and southern celery mosaic in Turkish tobacco plants<sup>a</sup>*

Hours <sup>b</sup> after inoculation	Virus used	Diameter <sup>c</sup> of primary lesions mm.	Systemic spread
24	Tobacco	none apparent	.....
24	Cucumber	" "	.....
24	Celery	1.4	none
30	Tobacco	none apparent	.....
30	Cucumber	0.3	none
30	Celery	2.0	"
48	Tobacco	0.75	none
48	Cucumber	2.0	"
48	Celery	4.0	"
60	Tobacco		.....
60	Cucumber	2.6	none
60	Celery	4.1	very slight
72	Tobacco	2.0	none
72	Cucumber	4.7	started
72	Celery	6.2 (indefinite edges)	in old and young leaves
96	Tobacco	2.7	in young leaves
96	Cucumber	6.3 (indefinite edges)	in old and young leaves
96	Celery	8.2 (some coalesced)	in old and young leaves
168	Tobacco	coalesced	in young leaves
168	Cucumber	"	in old and young leaves
168	Celery	"	in old and young leaves

<sup>a</sup> These data are the result of series of studies made by means of the Holmes technique (13) for detection of starch-retention patterns due to virus infection.

<sup>b</sup> Hours elapsed after inoculating leaves and placing plant in dark, cold (10° C.) room and later in dark, warm (22° C.) closet, in accordance with practice outlined by Holmes (13).

<sup>c</sup> After bleaching of chlorophyll and staining of starch, plants were dried and preserved. Diameter of lesions represents the average measurement of 10 lesions on each of the 3 leaves containing the largest number of lesions.

southern celery-virus lesions increased to over 3 times that of the ordinary tobacco-virus lesions. Measurements showed that celery-virus lesions also were slightly more than  $\frac{1}{4}$  larger than those of the common cucumber virus. These diameter differentials were consistent and typical.

First indications that the ordinary tobacco virus was escaping into surrounding tissues from the primary lesions was not noticeable until nearly 96 hours after inoculation. With the southern celery-mosaic virus, how-

ever, secondary spread was well started 60 hours after inoculation. It was not until nearly 72 hours after inoculation that the common cucumber virus had started its systemic spread. A more detailed analysis showed that after the first appearance of primary lesions systemic infection occurred in these experiments with celery virus in about 36 hours, with cucumber virus in about 42 hours, and with tobacco virus in about 66 hours.

Seventy-two hours after inoculation the greatest differences were notable in the action of the 3 viruses on young Turkish tobacco plants. Tobacco-virus lesions averaged 2.0 mm. in diameter, the disease was not systemic, and the plants appeared perfectly normal. Cucumber-virus lesions averaged 4.7 mm. in diameter, and iodine staining showed that systemic spread had commenced, though living plants showed no surface signs of disease. Celery mosaic-virus lesions averaged 6.2 mm. in diameter, systemic spread of the virus had gone through the plant, causing numerous distinct, chlorotic, vein-clearing patterns in the younger leaves, the plants were slightly stunted, and invaded leaves had taken on a crumpled and more than ordinarily pubescent appearance.

The results just reported for the southern celery and common cucumber viruses on Turkish tobacco were found to be essentially identical with those from similar studies made on *Nicotiana glutinosa*. This host also developed large whitish, canescent lesions at points of primary infection by the celery virus, becoming somewhat necrotic at about the time vein-clearing symptoms were well advanced. Besides being slower in producing vein-clearing symptoms, the cucumber virus did not produce observable primary lesions. As usual, ordinary tobacco virus produced typical small, dark necrotic lesions but no systemic infection.

Subsequent to the above observations on comparative time of appearance of infection and rate of spread of viruses in *Nicotiana* species, White Spine cucumber cotyledons were inoculated with typical celery, cucumber, tobacco, and tobacco-ring-spot viruses. This was done by wiping, as described in the foregoing. These experiments were repeated several times, and it was found that round, primary lesions developed from typical cucumber, celery, and ring-spot-virus inoculations, but no infections of any sort resulted from tobacco-virus inoculation. Exact time of appearance of lesions on cotyledons varied slightly. Generally speaking, it took from 3 to 4 days, though at times the celery virus caused primary lesions to appear towards the close of a 2-day period. Cucumber-virus lesions always appeared after celery-virus lesions, and ring-spot lesions developed last.

Southern celery-mosaic lesions on cucumber cotyledons have been described. Typical common cucumber-virus lesions are a little more marked in appearance than those produced by the southern celery-mosaic virus, producing larger indentations in the tissues on the under surface of cotyle-



dons. Tobacco-ring-spot lesions are much less marked, apparently not necrotic, and do not cause rapid collapse and drooping of cotyledons, as do the other 2 viruses. In one series of experiments measurements of lesions were made. With the celery virus the initial diameter averaged 1.3 mm., cucumber-virus lesions averaged 3.1 mm., and ring-spot lesions averaged about 3.2 mm.

Following cotyledonary inoculation of young cucumber seedlings with the typical ring-spot, celery and cucumber viruses, often the first symptoms to appear in the true leaves are numerous rounded chlorotic spots. Judging from time of appearance of such symptoms, cucumber and celery viruses become systemic within a day after primary lesions develop on the cotyledons. Ring-spot, on the other hand, remains more or less localized for from 2 to 3 days or even longer, before becoming systemic. It is also common during periods of high temperature for the celery virus to cause a watersoaked appearance of cucumber seedling hypocotyls followed by prostration and death of plants within a few days after inoculation. Under the same conditions this occurs also when seedlings are infected with the cucumber virus, but it usually becomes evident some time after the celery-virus-infected seedlings have collapsed. Such seedling collapse has not been observed in the writer's experimentations with the ring-spot carried on in parallel series with the cucumber and the celery viruses.

#### HOST-RANGE STUDIES

It already has been pointed out by Doolittle and Wellman (5) that southern celery mosaic is a typical virus disease of the mosaic type, is transmissible by artificial juice inoculations as well as by using the common melon or cotton aphid (*Aphis gossypii* Glov.), and is similar to common cucumber mosaic in its symptoms on certain hosts. In parallel series of experiments similarity of symptoms on cucumber seedlings of southern celery mosaic, common cucumber mosaic, and tobacco-ring-spot mosaic has just been pointed out. At times, symptoms of ordinary tobacco mosaic on Broadleaf tobacco and southern celery mosaic on the same host have marked resemblance. During the study of the southern celery-mosaic virus it was even considered possible that it might be the latent "healthy"-potato or tobacco-mottle virus. Host-range studies were instituted to determine relationships to these other viruses. Numerous series of plants of known susceptibility and reaction to definite viruses were grown and inoculated with southern celery mosaic and parallel series were inoculated at the same time and in identical manner with viruses with which comparison was desired.

### Legumes

Certain legumes were studied: Tennessee Green Pod, Golden Cluster, Red Valentine, Kentucky Wonder, White Marrow, Giant Stringless Green Pod, Improved Round Pod Valentine, and Refugee varieties of the common garden bean (*Phaseolus vulgaris* L.), and in addition, two other legumes, the Broad bean (*Vicia faba* L.) and the Black cowpea (*Vigna sinensis* Endl.). The garden beans were inoculated by the wiping method at different ages on both adult and juvenile leaves, and reinoculated once and in one series 3 times. No symptoms of celery-virus infection were found on any of the garden beans. Juices obtained from over a hundred plants, representing inoculated individuals of every variety, were used to inoculate cucumber cotyledons but no virus lesions or systemic infection resulted.

The Golden Cluster garden bean apparently was immune from the celery virus, though ordinary tobacco virus caused numerous typical primary lesions, such as have been described by Price (20). The Black cowpea was not infected by the celery virus and was not a symptomless carrier of the disease, since many inoculated plants were used as the source of inoculum but no virus was recovered. A parallel series inoculated with the tobacco-ringing-spot virus developed primary lesions and systemic infection typical of the disease as described by Price (21). *Vicia faba* developed small purplish primary lesions when inoculated with celery virus, but the disease did not become systemic.

### Celery

Mosaic symptoms on celery (Golden Self Blanching) in the field in Florida have been described by Foster and Weber (7) and discussed in more detail by Doolittle and Wellman (5). In the field the most striking symptoms are a yellowed condition of the foliage and apparent stunting of the plant (Fig. 1, A). On close inspection, irregular areas of pale green and yellowish color are found in leaf lamina similar to mosaic diseases on other hosts. These patterns often are brilliant, developing a mottled appearance that is quite characteristic of the disease. This is illustrated in figure 3, and is notable at times for its watered moiré-like effect. There is also sharp geotropic curvature of fleshy petioles and rachises of the compound leaves. These symptoms often are accompanied by buff, shrivelled, and sunken areas of these portions of the leaves (Fig. 1, A). Southern celery mosaic may cause some twisting and savoying of leaflets but apparently does not produce filiformities or other marked distortions.

Seedling celery plants, infected side by side with the southern celery-mosaic virus and the common cucumber-mosaic virus, differed somewhat in systemic symptoms, since (Fig. 4) the mottling produced was more brilliant in southern celery mosaic. At an early stage of infection, plants infected

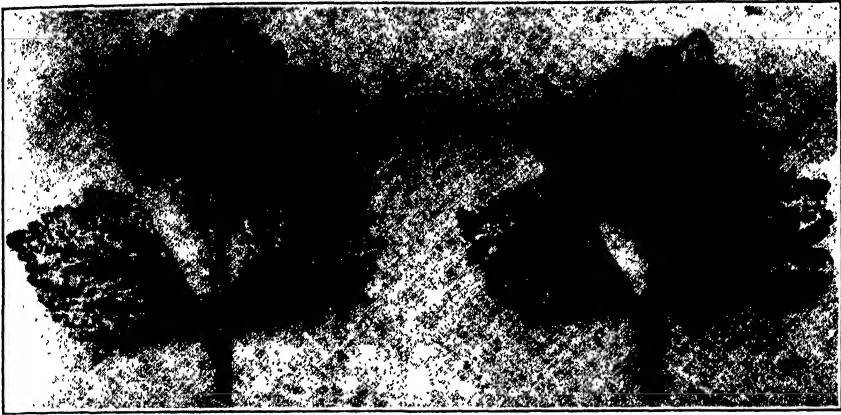


FIG. 3. Leaflets of nearly mature Golden Self Blanching celery plant, which show brilliant patterns of watered type characteristic of the southern celery mosaic. These leaflets came from a naturally infected plant growing near the spot where, 3 seasons before, the plant was obtained that furnished the original source of the southern celery-mosaic virus used in the virus-identification studies here reported. Compare with figure 5. B.

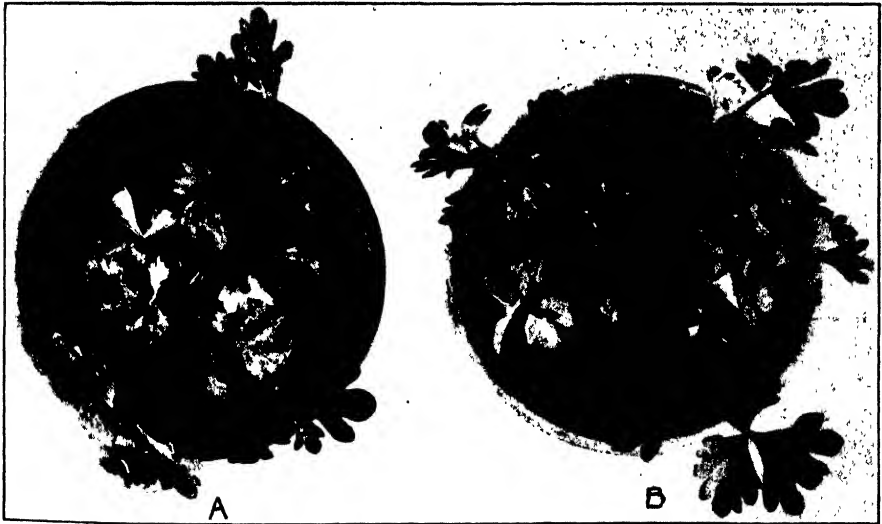


FIG. 4. Golden Self Blanching celery seedlings inoculated 9 days before the photograph was taken, showing the first systemic symptoms of infection by the virus of southern celery mosaic and the virus of common cucumber mosaic. A. First symptoms of southern celery mosaic, consisting of bright vein clearing and the beginning of a bright mottling pattern, and a downward curvature of leaf rachises and petioles that gives a stunted appearance to the plant. B. First symptoms of infection with the common cucumber-mosaic virus producing much milder mottling and vein-clearing patterns on the leaves, with less curvature of petioles and rachises.

with common cucumber mosaic and tobacco ring-spot showed symptoms similar to those of southern celery mosaic, except that chlorotic spots on leaflets and curvature of petioles and rachises were most marked in plants affected with the southern celery-mosaic virus. Leaves, on plants infected with common cucumber mosaic, after about a month, showed filiformities and raised blister-like areas following a mild mottling (Fig. 5, B). These leaves, moreover, became dark green and upright in growth habit rather than chlorotic and curved downwards, as in the case of southern celery mosaic. These symptoms produced by the common cucumber virus compare well with those described by Poole on celery; and the writer is convinced that Poole (19), Harvey (9), and Elmer (6) were working with common cucumber mosaic (*Cucumber virus 1*) on celery. Plants infected with tobacco-ring-spot virus for a month exhibited a pattern of small, green and yellowish spots, many of which contained small necrotic areas. Leaflets on these plants were also malformed, producing "shoe-string" effects, and the whole plant was more yellow than those infected with either the cucumber or celery mosaic.

### Cucurbits

Systemic symptoms of 3 mosaics have been studied on White Spine cucumber seedlings. Under certain environmental conditions as yet not fully understood, seedlings diseased with southern celery mosaic may not develop the hypocotyl collapse, prostration, and final death of the plant, such as has already been noted. If conditions at this critical period are such that individuals do continue growth, systemic mottling develops, and it is quite different in appearance from that produced by the viruses of the common cucumber or tobacco-ring-spot mosaics.

Cucumber seedlings, infected with southern celery mosaic, and that have survived the time when they usually die, are markedly stunted, the hypocotyls are so weak as to be unable to support the plants in a normal upright position, and the leaves display (Fig. 5) an indistinctly marbled or irregular moiré-like pattern, roughly suggestive of a dull yellow and green watered figure on cloth. The marked angular areas and more general chlorosis of true cucumber mosaic (*cf.* Doolittle (2)), and the definitely round-spotted effect accompanied by slight necrosis of tobacco-ring-spot mosaic (*cf.* Wingard (24)) are not the same as the southern celery-mosaic symptoms. Differentiation of these viruses, however, based on systemic symptoms on cucumber seedlings is often deceiving, since there are times when the symptom effects grade into one another most confusingly.

It should be noted, in connection with cucurbitaceous hosts, that southern celery mosaic is capable of readily infecting both Cocozelle and Summer Crookneck squashes, and also muskmelon and watermelon seedlings. In the



FIG. 5. Systemic symptoms of southern celery mosaic on cucumber and common cucumber mosaic on celery. A. Cucumber (White Spine) plant systemically infected with southern celery-mosaic virus for about 3 weeks. Note characteristic marbled or moiré-like mottled pattern on the leaves, and shortening of internodes. B. Leaf of celery (Golden Self Blanching) plant systemically infected with common cucumber mosaic virus for about six weeks. Note severe malformation of leaflets, which includes twisting, blister-like effects, filiformities, and thickened lamina. Compare the appearance of this leaf with figures 1, A and 3.

case of watermelons (Kleckley Sweet, Tom Watson, and Florida Favorite varieties were used) their susceptibility was especially interesting, since, according to Doolittle and Walker (4) as well as the writer, these hosts apparently are immune from common cucumber mosaic.

#### Emilia

A flowering plant, Flora's paint brush (*Emilia sagittata*), was found to be susceptible to ordinary tobacco (*cf.* Grant (8)), and the common cucumber and southern celery-mosaic viruses. All 3 viruses, used as inoculum under summer conditions at Arlington, Virginia, infected this host systemically and could be easily recovered from the infected leaves. The celery virus, however, produced no visible symptoms, while tobacco-mosaic-infected plants were severely mottled, and the cucumber virus caused a mild mottling.

#### Datura

In studying a possible relationship of southern celery-mosaic to the latent potato- or tobacco-mottle virus described by Johnson (14), parallel series of Jimson weed (*Datura stramonium* L.) seedlings were inoculated

with juice from celery infected with the southern celery-mosaic virus and with juice from potatoes infected with the tobacco-mottle virus. The plant showed a typical reaction to the tobacco-mottle virus resulting in characteristic reticulate patterns on leaves systemically infected. Inoculated leaves of Jimson weed plants, used to test the southern celery-mosaic virus, began dropping about a month after inoculation. They were characterized at that time by a few large, diffuse, whitish spots at points of infection. It was possible to recover the southern celery-mosaic virus from such leaves, but repeated trials of plant parts that had not been inoculated gave negative results.

### Peppers

Several varieties of garden peppers inoculated by the wiping method with southern celery-mosaic virus were all susceptible to it. Small seedlings of Tabasco pepper (*Capsicum annuum* var. *conoides*) developed black primary lesions on leaves inoculated with the celery virus within a period of 3 to 8 days. Moreover, the disease became systemic almost as soon as the lesions appeared, becoming first noticeable as a twisting or crook neck of the tender tip region of the stem. After a few days the crook straightened out; the plant remained living but severely stunted and marked mottling symptoms had developed on leaves systemically infected while young. When inoculated with the cucumber virus no infection was obtained. These plants were reinoculated, kept for a week, and the juice used as inoculum on cucumber seedlings; but, apparently, no common cucumber-mosaic virus was present.

Plants of a variety of sweet pepper (*Capsicum frutescens* var. *grossum*), California Wonder, were inoculated by wiping with the common cucumber-, southern celery- and ordinary tobacco-mosaic viruses. In series of 10 plants each the tobacco virus caused 10 to become diseased, the cucumber virus infected 7, and the celery virus infected 6. The ordinary tobacco virus was more rapid in its infection than the celery virus, producing systemic infection 3 to 7 days after inoculation. A typical black streak symptom developed on tender stems, and leaf petioles adjacent to streak marks on stems yellowed and dropped almost immediately after its appearance. The southern celery-mosaic and common cucumber-mosaic viruses were systemic 9 days after inoculation. With the celery virus a typical light brown streak appeared on sweet-pepper seedlings, causing as severe dropping of leaves as did the tobacco virus, and sometimes killed seedlings. Many plants also had the crook-neck symptom similar to the celery virus attack on Tabasco pepper. These plants soon straightened up and, although the plants were not always killed, the flowers dropped or did not mature fruits. Systemic mottling caused by the tobacco virus was severe, as was

mottling due to the celery virus. The cucumber-virus mottling apparently was of a milder type, though badly affected plants did not produce fruits. Stunting of growth was characteristic of the disease produced by all 3 viruses, though it varied somewhat in intensity, depending on point of inoculation.

#### Commelina

*Commelina nudiflora* has already been named and discussed as a host for southern celery mosaic (3, 5, 23) and is illustrated in figure 1, B. This host seems to be immune from infection by *Tobacco virus 1*. Thus far, attempts to inoculate it with the common cucumber-mosaic virus also have failed. The southern celery-mosaic virus does not infect *Commelina* when inoculations are made by the rubbing method. It appears to require insect transmission or the use of needle or hypodermic methods of inoculation to produce infection. The southern celery-mosaic virus, once it becomes systemic, exists from year to year without much effect on the normal behavior of the plant. Systemic infection is the rule; the mosaic patterns on leaves consist of yellowish green areas that are not angular but give a mildly marbled or watered and moiré-like appearance. It is only in exceptional cases that leaf malformations have been found, and there is apparently no death of tissues (Fig. 1, B.).

#### Tomato

Tomato plants (Globe), growing side by side and inoculated with the southern celery-mosaic, common cucumber-mosaic and ordinary tobacco-mosaic, exhibited symptom differences. The ease of infecting tomato seedlings by ordinary tobacco mosaic is known by students of this virus. These plants showed severe systemic infection with the ordinary tobacco mosaic within 3 to 4 days after inoculation. Of 10 plants inoculated, 10 were diseased. Under the same conditions, it took about 9 or 10 days for systemic infection (vein clearing) to appear on tomatoes inoculated with southern celery-mosaic and, of 10 inoculated, only 2 were diseased. The common cucumber-mosaic virus caused vein-clearing symptoms at about the same time as did the celery virus, and, of 10 plants inoculated, 4 were diseased.

The 3 series of plants were examined 30 days after inoculation. Ordinary tobacco-mosaic-infected tomatoes were badly stunted, the leaves were crumpled and much distorted and somewhat blistered, and they showed characteristic yellow and green mosaic mottling, typical of the disease. In some cases apparently complete inhibition of flower development occurred, and the flower buds did not open. Plants systemically infected with southern celery mosaic were less stunted than those affected by the tobacco mosaic. The leaves were marked with a reticulate pattern and mild

mottling but there was no distortion of leaf lamina. The flowers were few in number but appeared to be normal. The common cucumber-mosaic-infected plants were more stunted than those affected with the celery-mosaic virus, but not so severely shortened as ordinary tobacco-virus-infected individuals. However, plants infected with common cucumber-mosaic virus were distinctly characterized by much filiformity of leaves, accompanied with little mottling, and, although flowers were present, they were weak and dropping.

### Nicotiana Species

Plants of the genus *Nicotiana* were used as possible hosts to differentiate the celery, cucumber, and tobacco viruses. It has been demonstrated by Holmes (12) and others that *N. glutinosa*, when inoculated with ordinary tobacco-mosaic virus, develops typical small black lesions of diagnostic value. Parallel series of plants of this species were, therefore, inoculated by the wiping method with the common cucumber-, the southern celery-, and the ordinary tobacco-mosaic viruses. Typical lesions of ordinary tobacco virus appeared 2 days after inoculation with this virus but they did not enlarge greatly and the virus remained localized. The first symptom of southern celery-mosaic infection appeared on the plants 6 days after inoculation as numerous whitish, round spots, about 5 mm. in diameter on the inoculated leaves. The spots were not necrotic but became even whiter during the next few days. Such canescent spots were stained with iodine, as described by Holmes (13), and found to be primary lesions at a late stage of development. The first vein-clearing symptoms appeared on plants 7 days after inoculation and, a day later, systemically infected leaves were more yellowed and had a somewhat blanché appearance. Four days after, vein-clearing, necrotic, zigzag lines started to develop on these leaves and primary lesions also began to be necrotic. Plants inoculated with common cucumber mosaic did not produce canescent primary lesions, and appearance of vein clearing occurred a day later than with the celery virus. Systemic mottling and chlorosis were not so serious on the cucumber-virus-infected plants and no necrotic effects developed.

Symptoms on Turkish and Broadleaf tobacco (*Nicotiana tabacum*) seem to be almost identical when infected with the common cucumber virus or with the southern celery-mosaic virus. Primary lesions of the whitish type described on *N. glutinosa* are produced by the southern celery-mosaic virus on both Turkish and Broadleaf tobacco. Old lesions of this type are illustrated in figure 6. No primary lesions were noticed in connection with the common cucumber-virus inoculations. Upon systemic infection of the 2 tobaccos, southern celery mosaic causes vein clearing, followed by a more yellowish appearance and mild mottling of the plants, coupled with



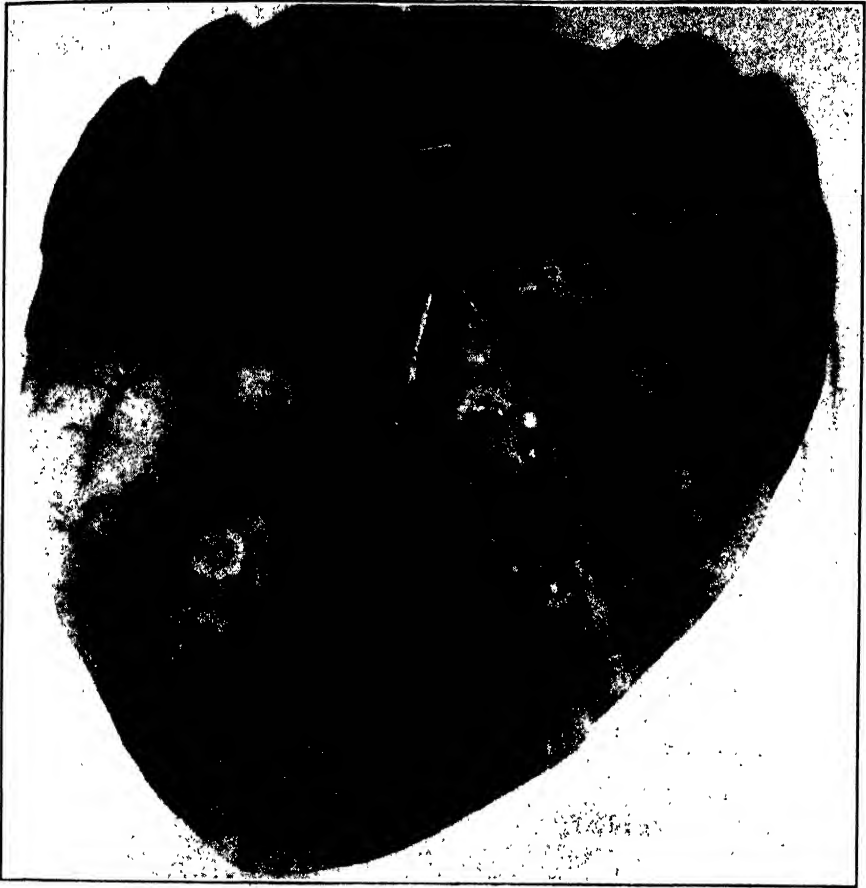


FIG. 6. A lower leaf of a Broadleaf tobacco plant, inoculated with the southern celery-mosaic virus, 10 days before this picture was taken. Virus infection has resulted in the production of numerous canescent lesions a few of which, as the infection grows older, are becoming somewhat necrotic. Note that in some lesions on the right side of the leaf chlorophyll has come back into what was once very light-colored spots.

crumpling of the leaves downward and what seems to be a greater pubescence on such leaves. These latter symptoms are quite marked in contradistinction to the upright orientation of leaves systemically infected with the common cucumber virus, which also produces a gray type of chlorosis, spotted with a few large, dark green areas. As the diseased plants become older, differences in appearance are less marked until, at the time of blossoming, it is almost impossible to see specific differences in symptoms. Old plants, especially of the Broadleaf tobacco, affected with either the celery or the cucumber virus, often resemble to a marked degree old plants affected with the ordinary tobacco mosaic.

## Susceptible Host List

As has been indicated, studies leading to fairly complete data on plant species susceptible to the southern celery-mosaic virus are in progress. Thus far, however, 2 classes and 8 families of plants represented by 23 species and varieties have been listed as susceptible and are as follows:

## MONOCOTYLEDONAE

## COMMELINACEAE

*Commelina nudiflora* (creeping day flower or wild wandering jew)

## DICOTYLEDONEAE

## CHENOPODIACEAE

*Beta vulgaris* (red garden beet)

## LEGUMINOSAE

*Vicia faba* (Broad bean)

*Vigna sinensis* (Black cowpea)

## UMBELLIFERAE

*Apium graveolens* (celery)

## HYDROPHYLLACEAE

*Phacelia whittlavia* (California bluebell)

## SOLANACEAE

*Nicotiana glutinosa* L.

*N. tabacum* (Broadleaf tobacco)

*N. tabacum* (Turkish tobacco)

*Datura stramonium* (Jimson weed)

*Lycopersicum esculentum* Mill. (Globe tomato)

*Capsicum annuum conoides* Irish (Tabasco pepper)

*C. annuum grossum* Sendt. (sweet pepper)

*Physalis pubescens* L. (common ground cherry)

*P. lagascae* R. & S. (wild ground cherry)

## CUCURBITACEAE

*Cucurbita pepo* var. *condensa* Bailey (Cocozelle squash)

*C. pepo* var. *condensa* (Summer Crook-neck squash)

*Cucumis sativus* (White Spine cucumber)

*C. melo* (muskmelon)

*Citrullus vulgaris* Schrad. (watermelon)

## COMPOSITAE

*Zinnia elegans* (zinnia)

*Tagetes patula* (French marigold)

*Emilia sagitata* (Flora's paint brush)

## DESCRIPTION OF CELERY VIRUS 1

Name: Southern celery mosaic (*Celery virus 1*)

Type: This publication, figures 1, A and B; 2, A; 3; 4, A; 5, A; 6.

Host Families: Wide range, families so far listed include Commelinaceae, Chenopodiaceae, Leguminosae, Umbelliferae, Hydrophyllaceae, Solanaceae, Cucurbitaceae, and Compositae.

Differential Hosts:

*Apium graveolens* (Golden Self Blanching celery), severe chlorosis; downward curvature of rachises and petioles; discoloration and

shrivelling of rachises and petioles; leaflets not reduced in number, no filiformity nor upright orientation.

*Cucumis sativus* (White Spine cucumber), sunken lesions on underside of cotyledons; collapse of hypocotyl and death of seedlings more rapid than with *Cucumber virus 1*; watered, moiré-like pattern on leaves of seedlings escaping death.

*Datura stramonium* (Jimson weed), diffuse primary lesions on leaves; not systemic; infected leaves drop.

*Capsicum annuum* var. *conoides* (Tabasco pepper) black primary lesions; necrosis of stem and crook neck; stunting and mottle.

*Commelina nudiflora*, no primary lesions; systemic mottling of moiré-like pattern; no necrosis.

*Lycopersicum esculentum* (Globe tomato), difficult to infect; mottling mild and may be reticulate pattern; no severe distortion of leaf lamina.

*Nicotiana glutinosa*, distinct, canescent primary lesions, becoming necrotic; yellowish blanched systemic symptoms; necrotic, zigzag patterns on systemically infected leaves.

*Nicotiana tabacum* (Broadleaf tobacco), canescent primary lesions becoming necrotic; brilliant vein clearing; chlorosis and downward crumpling of leaves; more than ordinarily pubescent appearance; and zigzag necrotic patterns.

Resistance to Aging *in vitro*: 6 to 8 days at room temperature.

Resistance to Desiccation: 72 hours on cheesecloth, killed when leaf tissues are dry enough to crumble.

Thermal Death Point: 75° C., 10 minutes.

Resistance to Chemicals (15 minutes): resists 50%  $C_2H_5OH$ ; killed by .02%  $HNO_3$ , .1% NaCl, and 50% sat. sol.  $Ca(OH)_2$ .

Resistance to Dilution: variable, maximum 1:100,000.

Cytological Characters: no inclusion bodies; suppressed chloroplast development; shortened palisade cells; compact parenchyma.

#### DISCUSSION AND CONCLUSIONS

The studies herein reported adequately establish the identity of the virus causing the southern celery mosaic. Besides causing a disease that results in increasingly serious losses in celery, the virus has been observed by the writer in the field as very destructive on other truck crops, *e.g.*, cucumbers, Cocozelle squash, summer crook-neck squash, and sweet or bell peppers. The host range is wide and is still being studied, and, in addition to hosts mentioned in this paper, the virus is able to infect numerous other weeds, ornamentals, and truck-crop plants.

The writer has always found, in Florida, that, when plants are affected in the field with the southern celery-mosaic virus, the disease is traceable largely to the presence of a single weed, *Commelina nudiflora*, called creeping day flower or wild wandering jew. In some cases, for instance isolated fields of diseased peppers, it has not been easy to follow the connection, but always, when locations of seed beds were known and detailed surveys

of field edges were made, mosaic infection of crops could be traced to natural insect inoculation from the weed host. Yet, it should be remembered that other plants than *C. nudiflora* may act as reservoirs of the disease. It has been pointed out by Doolittle and Wellman (5) that celery with systemic symptoms apparently identical with southern celery mosaic were found in plants from Ohio, Wisconsin, New York, and California. Notwithstanding the fact that it has been reported in northern States, it is my opinion that southern celery mosaic is of subtropical or tropical origin and is primarily a disease of *C. nudiflora* that does not withstand severe spells of cold weather. This belief early led the writer to consider naming the celery-mosaic trouble, just described, Commelina mosaic (*Commelina virus 1*). Had this been done the economic side of the subject, its attack on an important crop plant, might have been obscured in later studies and it is too important to be lost sight of.

*Celery virus 1* is not the same as the tobacco ring-spot studied in detail by Henderson and Wingard (10), although the properties of these 2 viruses somewhat resemble each other. The southern celery-mosaic virus will infect tomatoes when inoculated by the wiping method, whereas tobacco ring-spot apparently is incapable of this. On the other hand, the Black cowpea is immune from the southern celery-mosaic virus and tobacco ring-spot causes primary and systemic infection. On tobacco, cucumber, and celery plants infections with the 2 viruses cause distinctly different symptoms.

The ordinary tobacco mosaic, *Tobacco virus 1*, Allard, described by Johnson (15) and Allard (1), is quite distinct from *Celery virus 1*. The celery virus withstands aging *in vitro* only a few days, whereas tobacco virus withstands it for years. The tobacco-virus thermal death point is approximately 15 to 20 degrees C. higher than that of *Celery virus 1*. Cytological studies disclosed no cell-inclusion bodies in tissues infected with southern celery-mosaic virus such as those characteristic of ordinary tobacco mosaic. The celery virus infects cucurbits, which are immune from *Tobacco virus 1*, and becomes systemic in *N. glutinosa* on which the tobacco virus produces small, black, necrotic, primary lesions but no systemic infection.

Southern celery mosaic (*Celery virus 1*) is similar in some ways to cucumber mosaic (*Cucumber virus 1*, Doolittle) described by Doolittle (2), Johnson (15), and Doolittle and Walker (4) in its properties and host relationships. It may be that these 2 viruses are fairly closely related. The thermal death point of *Celery virus 1* is only about 5° C. higher than *Cucumber virus 1*, which is not a greatly significant difference. The cucumber virus withstands aging *in vitro* about 3 days, whereas the celery virus withstands the treatment 6 to 8 days. On the other hand, watermelons are immune from common cucumber mosaic but are readily infected by the southern celery mosaic. The celery virus causes nonnecrotic, whitish or

canescent, primary lesions on *Nicotiana glutinosa* and *N. tabacum*, followed by necrotic zigzag patterns upon systemic infection; but the common cucumber virus produces no such effects. Filiformities characterize leaves of celery and tomato plants infected with *Cucumber virus 1* but are not induced by *Celery virus 1*. Celery virus infection on cucumber cotyledons results in smaller primary lesions than those produced by the cucumber virus; it may cause collapse and prostration of most cucumber seedlings a few days after infection, while *Cucumber virus 1* usually causes this some days later. Cucumber seedlings that survive prostration and death due to infection with *Celery virus 1* develop systemic infection, which results in a moiré-like or watered mottling pattern in the leaves, while systemic mottling, due to common cucumber mosaic, is characterized by more angular spots and is generally more chlorotic.

*Celery virus 1* is not latent-potato- or tobacco-mottle virus, for this virus, according to Koch (17), withstands 28 days' aging *in vitro*, which is 3 weeks longer than the celery virus can endure. Symptoms caused by southern celery-mosaic infection of tobacco are not like those of tobacco mottle. On *Datura stramonium*, *Celery virus 1* causes definite primary lesions, accompanied by dropping of inoculated leaves, but no systemic infection, and the tobacco mottle causes typical systemic symptoms in *D. stramonium*.

*Celery virus 1* is a new virus, and is the first celery virus to have been fully described. It is believed upon careful study of symptoms described in reports of celery mosaics by Poole (19), Elmer (6), and Harvey (9) that these investigators were working with common cucumber mosaic (*Cucumber virus 1*, Doolittle). Mosaic of celery occurs in the northern celery growing areas, but ordinarily differs in symptoms from that that is the subject of this paper. For these reasons, and because the disease was so long investigated in the South and is in so many cases traceable to the presence of the subtropical and tropical weed host, *Commelina nudiflora*, it has been named "Southern celery mosaic" and the virus has been given the specific designation *Celery virus 1*.

#### SUMMARY

A new virus, which causes a disease of true mosaic type on celery, is here described. The virus is transmissible by *Aphis gossypii* and artificial juice inoculations. It is filterable through a bacteria-excluding Berkefeld, "W," filter. It is destroyed upon heating for 10 minutes at 75° C. Freezing does not destroy it. Viruliferous juices may be diluted with water varying in ratios from 1:500 to 1:10,000 and occasionally at 1:100,000. Such solutions still remain infective, dependent on host plants and environmental conditions. The virus withstands aging *in vitro* 2 days at 28° C.,

from 6 to 8 days at 18° to 20° C., and remains infective at least 14 days when stored in a frozen condition. It withstands drying on pieces of cloth for between 3 and 5 days, in infected leaves it is destroyed within 1 day after they are dry enough to crumble but may last 2 days in dried cucumber cotyledons. It withstands 50 per cent ethyl alcohol for 15 minutes, but 1 per cent nitric acid, 50 per cent saturated hydrated lime solution, and 10 per cent sodium chloride solutions all destroyed the virus when treated 15 minutes. Cytological studies were made of diseased leaves of several hosts, but no cell-inclusion bodies of pathological nature were found. The spread of viruses in hosts was studied and it is characteristic of the celery-mosaic virus being described that it spreads more rapidly than the ordinary tobacco-, the common cucumber-, or the tobacco ring-spot viruses.

Symptom studies of virus diseases of the mosaic type have been made on many hosts comparing ordinary tobacco mosaic, common cucumber mosaic, tobacco ring-spot mosaic, latent or healthy potato (tobacco mottle) mosaic, and the southern celery mosaic. Differences have been discovered in the symptoms of the various virus diseases produced on a number of hosts, although it is to be noted that their intergradations in many cases make differentiation on a purely symptom basis dangerous criteria of specificity of causal viruses. However, a comparative analysis by combining symptomological and property studies of the viruses discloses that the virus originally recovered from celery is new and distinct.

It has been shown that the celery-mosaic diseases reported by Poole (19), Elmer (6), and Harvey (9) probably were due to the common cucumber virus (*Cucumber virus 1*, Doolittle) attacking celery.

Host-range studies, which are not completed, show that *Celery virus 1* has caused disease in plants of 23 species and varieties representing 8 families in both the Monocotyledonae and the Dicotyledonae. In addition to serious losses on celery in the field, this virus also has been found on other truck crops, notably Cocozelle squash, summer crook-neck squash, peppers, and cucumbers.

This virus is new, was first studied on celery where it causes a destructive mosaic disease, and has as its principal wild host the subtropical and tropical weed *Commelina nudiflora*. For these reasons, the author has named the virus *Celery virus 1* and has given the disease the common name *southern celery mosaic*.

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# NONPARASITIC LEAF SPOTS OF BARLEY<sup>1</sup>

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In some years, in Minnesota, leaf spots of barley, apparently of non-parasitic origin, are more prevalent than those caused by parasites and may become severe enough on some varieties to cause complete killing of foliage and premature ripening. Velvet, Glabron, Trebi, and other varieties of barley commonly grown in Minnesota frequently develop severe spotting from the seedling to adult stage on certain kinds of soil. Local areas or entire fields may be involved. Such spotting appears to be associated with malnutrition. On most varieties, however, nonparasitic spots are perhaps of little economic importance, except insofar as they furnish an avenue of entrance to semiparasitic organisms.

Although these nonparasitic diseases have been recognized for a number of years in Minnesota, it has been difficult to obtain definite information on symptoms, since leaf spots caused directly by fungi, *i.e.*, *Helminthosporium sativum* P. K. and B., *H. teres* Sacc., and similar congeneric species, also are usually abundant on barley in this State. Furthermore, in some seasons the symptoms of nonparasitic spots may be greatly modified by secondary fungi and bacteria. It has been shown, however, that spot-blotch organisms require considerable moisture in order to produce good foliage infection (9). The growing seasons 1930 to 1933, inclusive, have been ideal for the study of nonparasitic diseases in Minnesota, because of the relative lack of precipitation. In these years, practically no spot blotch or other *Helminthosporium* spots developed on plots of barley containing more than 125 varieties, even when they were sprayed repeatedly with a suspension of spores of parasitic fungi. But, on these same plots, many of the varieties developed certain types of nonparasitic leaf spots.

Recently nonparasitic diseases of cereals, especially of oats, have received particular attention in Europe. In 1930, Ferdinandsen and Winge (3) described a nonparasitic leaf spot of oats, which they named heritable blotch leaf, resembling one type of lesion observed by the writer on barley. Several workers (4, 6, 8) have found that magnesium deficiency in certain types of soils may result in mottling and chlorosis of oats and barley and other crops, while others (5, 7) have reported "yellow tip" or "white tip" of cereals, which can be controlled by the application of copper sulphate to

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the soil. These nutritional lesions appear, however, to be quite different from those commonly observed on barley in Minnesota.



FIG. 1. Types of nonparasitic spots on different varieties of barley. A. *H. d. deficiens* selection. B. Black Hull-less C. I. 666. C. Gorak, C. I. 1086. D. C. I. 2208. E. N. S. Wales selection. F. *H. d. decortiatum* C. I. 2230. G. Lion C. I. 923. H-K. Variation in symptoms on *H. d. decortiatum*.

In some years, it is almost impossible to differentiate between true spot blotch caused by *Helminthosporium sativum* and some of the nonparasitic spots or blotches of barley, especially if the two types are found on the same variety (Figs. 1-4). Clear differentiation is of considerable importance in studies on the epidemiology of *Fungi Imperfecti*, of inheritance of reaction to leaf spot, and also in the study of varietal resistance to disease. For this reason, observations and experiments were made in the field and greenhouse on many varieties of barley<sup>2</sup> to obtain information on symptoms, possible causes, and methods of control of the nonparasitic leaf spots.

<sup>2</sup> Most of the varieties of barley used in these tests were obtained from the Division of Agronomy and Plant Genetics, University of Minnesota, and M. N. Pope, Division of Cereal Crops and Diseases, U. S. Department of Agriculture.

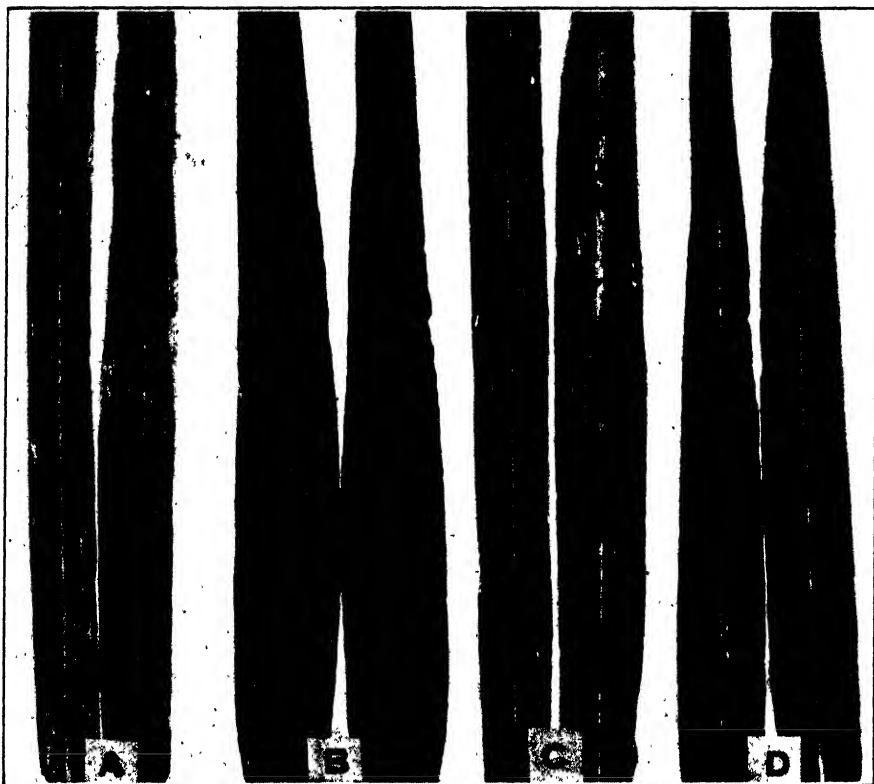


FIG. 2. Four types of nonparasitic spots on four different varieties of barley. A. Purple nudum, C. I. 1219. B. Zumpakei C. I. 692. C. *H. d. nigrilaxum* selection. D. N. S. Wales selection.

#### SYMPTOMS

The first evidence of the nonparasitic spots is generally the appearance of chlorotic, yellowish, or light brown areas, while the lesions produced by parasites usually begin as more or less water-soaked areas. These nonparasitic lesions can be distinctly seen by transmitted light and usually become much darker with age, varying from light brown to black. In some cases the lesions are distinct spots; in others there is a stripe effect similar to true barley stripe; and, occasionally, the entire leaf blade is bleached or discolored. There is considerable variation in depth of color under different environmental conditions, sunlight and high temperature being conducive to pigmentation. The disease area may be uniform in color throughout and sharply delimited from healthy tissue. In some varieties the outer edges of the lesion were marked by a narrow and much lighter zone (Fig. 1, B). In other cases spots were more or less diffused, so that it was almost impossible to separate diseased from healthy tissue, the effect being that of



FIG. 3. Barley plants, Yanehadaka C. I. 693, affected with leaf spots of non-parasitic origin.



FIG. 4. Leaves from 3 varieties of barley artificially inoculated with *Helminthosporium sativum*. A. Minsturdi, C. I. 1556. B. Lion, C. I. 923. C. Black Hull-less selection.

bronzing and scalding (Fig. 2, A). The size of lesions may vary greatly, ranging from small freckle-like spots (Gyanste, C. I. 654) through all gradations to the large blotch-like lesions in such varieties as Lion C. I. 923 and Trebi C. I. 936. Sometimes two or more different types of lesions occur on the same plants (see figures 1-3 for variation in lesion on leaves).

Another type of lesion, fairly common on such varieties as Hanna C. I. 203, and Steigum C. I. 907, resembled true barley stripe caused by *Hel-*

*minthosporium gramineum* Rab. It was difficult sometimes to distinguish "false stripe" and true stripe, for the symptoms of the former varied from indefinite chlorotic lesions, which turn brownish with age, to the ribbon-like lesions characteristic of true stripe.

Symptoms sometimes deviate from those of the spots or the "false stripe," and the entire leaf tip is bleached or discolored, as in White Hull-less Selection. In Black Hull-less C. I. 708 the characteristic symptom is blackening of the flag leaf.

The time of appearance of the nonparasitic lesions varies with varieties. On some, such as Zunpakei C. I. 692 and *H. d. decorticatum* C. I. 2230, the lesions consistently appear in the seedling stage, although on most varieties they do not appear to a great extent until the plants have reached the jointing or boot stage. In fact, the spots usually develop most rapidly after the heading of barley.

The spotting usually, but not always, progresses from the lower to the upper leaves, the lower ones often being most severely injured. Spotting develops uniformly and simultaneously on nearly all the plants of the same variety that are in the same stage of development.

The spots may be scattered, as in the variety New South Wales Selection, or aggregated, as found in Kamet Mugi C. I. 2253. In fact, parts of leaves may be completely destroyed as a result of coalescence of numerous spots (Tatien C. I. 665), or they may become so weakened that they dry up or die from the attack of secondary organisms (Gorak C. I. 1086 and Cornutum C. I. 724). The spots produced on a given variety were similar in successive years. The characteristic symptoms, also, have been observed on many varieties when grown in the greenhouse. In most cases, however, the lesions were more distinct and larger in the field than in the greenhouse. In general, the type of spots produced is characteristic for the variety concerned, although a number of varieties may develop similar types.

#### EXPERIMENTS TO DETERMINE CAUSAL FACTORS

##### Failure to Isolate Pathogen

Numerous tests of many different sorts indicated that these leaf spots are, apparently, of nonparasitic origin and are either inherent or nutritional maladies. Many attempts were made to establish a parasitic origin but none were successful. Field and greenhouse tests indicate that healthy plants never become spotted by contact with badly spotted plants, even though their leaves and culms may be interlocking.

Attempts were made repeatedly to isolate causal organisms. Diseased leaves from many varieties grown in the field or greenhouse were plated out in the usual manner on nutrient agar. The results were always negative, except when leaves bearing old lesions were selected. From such material

different species of fungi and bacteria were isolated occasionally, but in every case they appeared to be of secondary importance. Likewise, leaves with newly formed leaf spots failed to develop fungi when placed in moist chambers.

Sections, mostly free-hand, but some microtome sections, were prepared from many different types of lesions obtained from a number of sources. Some of the cells in diseased lesions were collapsed, discolored, and dead, and in many of the adjoining cells there was a scarcity of chloroplasts. The progressive killing of the cells was observed microscopically but no causal organism was detected.<sup>3</sup>

In order to determine whether the different types of leaf diseases would be transmitted through the plant juices, extracts of leaves from 7 diseased varieties were injected with a hypodermic syringe into apparently healthy plants of 12 different varieties. The varieties of barley were sown late in May in 9 successive series. The leaves from the respective varieties were thoroughly macerated by mixing with quartz sand and grinding in a mortar. The desired quantity of sterile water was then added and the extract for inoculation was obtained by filtering. Distilled water and juice from a healthy variety were injected into 2 similar series used as checks. On June 22, the date of injection, the plants varied in development from the jointing to the early boot stage.

The nonparasitic diseases could not be induced by any of the above methods. In no case did any of the plants injected with juice from diseased leaves develop more lesions than noninoculated plants or the plants injected with sterile water or with juice from healthy leaves.

The environmental conditions certainly were favorable for the development of the disease, since on the control plots near-by, which, however, were planted earlier, the lesions increased very rapidly during this period. Furthermore, the conditions also were favorable for bacterial development. Some of the leaves of one variety, Yanehadaka C. I. 693, used in making juice extraction were infected with *Bacterium translucens* var. *undulosum* S. J. & R., and the 12 varieties injected with the juice developed the bacterial disease.

*Effect of Cultural Conditions.* The nonparasitic diseases seem to develop best during late spring and early summer, both in field and greenhouse. In 1931 and 1932, 125 varieties of barley were sown in replicated plots in April and again in late August. As already pointed out, in both years severe spotting occurred on many of the spring-sown varieties; but almost none on late-sown barleys. Similar results were obtained also in the greenhouse tests.

<sup>3</sup> The writer is indebted to T. W. Graham, a graduate student in Plant Pathology, for his efficient help in histological studies.

Most of the varieties of barley sown in the fall and winter in the greenhouse failed to develop typical leaf spots. When these varieties, however, were sown in the field or greenhouse in the spring they became severely spotted. The same response of the many varieties in 2 successive years suggested that the cause might possibly be a temperature relationship. But the greenhouse test with 10 varieties of barley grown at 2 distinctly different temperatures, ranging from about 16° to 22° C., and from about 20° to 30° C., respectively, failed to produce sharp differences in degree of spotting. Lesions produced at the high temperatures were frequently darker in color than those at low temperatures. Several similar tests with fewer varieties were made, but they also were negative.

In order to determine whether lack of moisture could induce the development of disease, 7 susceptible varieties of barley were sown in quadruplicate in porous pots. Two pots of each variety of barley were put in a large, flat pan in which the water was maintained at a level of 1-2 inches from the bottom. The other set of pots was watered just enough to keep the plants from wilting. No differences in degree of spotting between the 2 series was discernible at the end of the test.

*Effect of Soil Type.* Field observations indicate that the nonparasitic diseases develop on a wide range of soil type. In order to obtain definite information, greenhouse tests were carried out under controlled conditions.

In the spring of 1932 duplicate 4-inch pots of 25 different varieties of barley were sown in 4 types of soil; black loam, sandy soil, muck, and peat. These pots were all placed in a pit in the greenhouse and their positions were altered from time to time so as to give every plant as nearly as possible

TABLE 1.—*The comparative severity of nonparasitic lesions on 9 different varieties of barley when grown in the greenhouse on four different soil types. 1932*

Variety and C. I. No.	Type of soil and severity of disease <sup>a</sup>			
	Black loam	Sandy soil	Muck	Peat
Abyssinian C. I. 1216 .....	H-L <sup>b</sup>	H	H	H
Algerian C. I. 1179 .....	0	0	—	0
Black Hull-less C. I. 666 .....	L	L	L	L
H. d. decorticaturn C. I. 2230 .....	H -	H	H	H
Lion C. I. 923 .....	H -	H	L	L
New South Wales, Selection .....	L +	H	H	H
Peatland, Minn. 452 .....	0	M	L -	0
Svanhals, Selection .....	T	M	L -	0
Trebi C. I. 936 .....	M	L	H	—

<sup>a</sup> Notes were taken at boot or heading stage.

<sup>b</sup> H = heavy spotting; M = moderate; L = light; T = trace.

similar environment. The 9 varieties listed in table 1 are representatives of the 25 varieties on the different kinds of soil.

Twenty-four out of 25 varieties developed necrotic lesions somewhat similar to those observed in the field. There was considerable variation in the severity of the disease on certain varieties of barley on different types of soil. In general, the disease appeared to be most severe on the sandy soil and least severe on peat. It should be pointed out, however, that 8 varieties produced equally severe spotting on all 4 soils, but 8 other varieties developed more spotting on sandy soil than on the other types of soil. Some varieties developed decidedly more spotting on muck than on peat. Others were severely damaged by leaf spots on all 4 soil types and 1 remained clean.

In another test, in 1932, duplicate pots of 3 varieties were grown for 2 months on the following types of soil: peat, Clyde silt loam, Merrimac loamy fine sand, Gloucester fine sandy loam, Fargo clay, and a very rich garden soil. Notes were taken when the plants were in the jointing stage. In this test there was very little difference in the response of the varieties on the different soil types. Trebi, however, became much more heavily spotted on the Merrimac loamy fine sand than on the other 5 types. Severe leaf spotting occurred on a selection of *H. d. decortioatum* grown on 6 different soil types, while Gorak grown on rich garden soil was practically free, but developed a trace of spotting on the other soil types. In a later test in the spring Gorak became severely spotted. Thus, it would seem that the prevalence of lesions on a variety is not always governed by the same set of factors. Undoubtedly, the spotting on certain varieties is an expression of genetic factors as in Abyssinian C. I. 1216 and Yanehadaka C. I. 693 (Fig. 3).

*Effect of Salts.* A number of workers have shown that the presence or absence of certain salts may prevent or induce nutritional diseases (4, 5, 6, 7, 8). Therefore, the writer, in a preliminary way, tried the effect of adding one or more salts of aluminum, ammonium, barium, calcium, copper, iron, lead, lithium, magnesium, manganese, potassium, silver, boron, and zinc to the soil. Of the 20 compounds used only one, sodium borate, produced distinct spotting, although some compounds burned and even killed the leaves or plants. The spots produced by sodium borate and boric acid were similar to some of the nonparasitic spots and bore some resemblance to true spot-blotch lesions. The production of distinct lesions by the addition of boron to the soil is of considerable interest, since Eaton (2) is of the opinion that boron increases susceptibility of barley to spot blotch, *Helminthosporium sativum*, but renders it resistant to powdery mildew, *Erysiphe graminis* DC.

In order to test still further the toxicity of 16 compounds, freshly cut



culms of Minsturdi and Glabron barley were placed in Erlenmeyer flasks containing 5 different concentrations of each compound. As in soil tests, the boron compounds were the only ones that produced definite necrotic spots.

In a number of other tests it was shown that a trace of boron, either as boric acid or borax, could cause spot-blotch-like lesions. In fact, water cultures containing 1 part of boron in 10,000,000 produced numerous



FIG. 5. Lion, C. I. 923 and Trebi, C. I. 936 barley with boron leaf spots produced by adding 0.21 gm. of boric acid to 2,000 gms. of soil.

necrotic lesions on the leaves of healthy barley in 3 or 4 days. Higher concentration of boron produces similar effects but in proportionately shorter time. The type of symptoms produced by boron varies somewhat with amounts applied, stage of host, variety concerned, and humidity and temperature involved. The boron spots usually appear first on the lower leaves and on the tips of young vigorous growing leaves, especially along the margin. The spots may become so numerous that the leaves are destroyed, or even the entire plant may be killed. (Fig. 5). The boron spots that resemble most nearly the lesion produced by *Helminthosporium sativum* or other species of that genus are those produced on the leaves that continue to grow after the application of the boron. In many respects, though not in color, boron injury resembles the injury frequently observed on many of our common varieties of barley throughout Minnesota, especially on soil where barley does not thrive.

In still another test 11 varieties of barley were grown in 7-inch pots, each containing approximately 2,000 grams of garden soil. To one series of duplicate pots was added 50 grams of lime just preceding the sowing of seed. In two other series boric acid and copper sulphate solution were added, respectively, at 4 different times during seedling and jointing stages. The total amount of copper sulphate or boric acid solution given each pot was only .21 gram. Notes were taken when plants were in the jointing or

TABLE 2.—*The effect of applying solutions<sup>a</sup> of lime, copper sulphate, or boric acid to the soil in 7-inch pots in which were grown 11 different varieties of barley*

Variety and number	Treatment per pot and degree of spotting <sup>b</sup>			
	Control	Lime 50 gms.	CuSO <sub>4</sub> .21 gms.	Boric acid .21 gms.
Algerian C. I. 1179 .....	0	0	0	M <sup>c</sup>
Black Hull-less C. I. 878 .....	T +	L -	L -	M
Gorak C. I. 1086 .....	M	M	L +	H +
H. d. decortiatum C. I. 2230 .....	H	H +	H	H
H. d. deficiens, Selection .....	L -	L -	L -	H
H. v. duplinigrum C. I. 2206 .....	0	0	0	0
Lion C. I. 923 .....	T	T	T	H
Minsturdi, Minn. 437 .....	T +	T +	T +	H
New South Wales, Selection .....	L	L	L	H
Purple Nudum C. I. 2250 .....	M	M	M	H +
Trebi, C. I. 936 .....	T	T	T	H +

<sup>a</sup> Each pot contained about 2000 gms. of soil. The lime was applied at time of sowing, but the boric acid and copper sulphate solutions were applied at 4 different times during the growing period of the barley.

<sup>b</sup> The notes were recorded at jointing or boot stage.

<sup>c</sup> H = heavy spotting; M = moderate; L = light; T = trace.

TABLE 3.—*The effect of applying boric acid to the soil on the development of boron spots and powdery mildew on barley in the field and greenhouse*

Amount of boric acid per square meter	Variety and degree of infection or spotting <sup>b</sup>										
	Glabron			Manchuria			Minsturdi				
	Boron spots		Powdery mildew	Boron spots		Powdery mildew	Boron spots		Powdery mildew	Green-house	
	Field	Green-house	Field	Green-house	Field	Green-house	Field	Green-house	Field		
10 gms. . . . .	M	H	L + <sup>c</sup>	H	M +	H	L + <sup>c</sup>	H	M +	T - <sup>c</sup>	—
20 gms. . . . .	H	H +	T	H	H -	H +	T	H	H	0	—
Control .. .....	0	0	M +	H +	0	0	M +	H +	0	T	—

<sup>a</sup> Boric acid was applied when the plants had developed 3 to 4 leaves.<sup>b</sup> H = heavy infection or spotting; M = moderate; L = light; T = trace.<sup>c</sup> The barley plants on the plots receiving boric acid were stunted, many of the leaves and even the plants were killed by boron injury; hence the density of foliage was much less than in the controls. Plants used in the greenhouse were "boron-diseased" plants transplanted from the field.

boot stage. The results are presented in table 2. As in previous tests, boric acid greatly stimulated production of necrotic lesions. All varieties tested were susceptible to boron injury. Lime and copper sulphate did not decrease or increase the amount of physiologic spotting over the controls.

#### RELATION OF BORON SPOTS TO HELMINTHOSPORIUM AND POWDERY MILDEW

A number of different tests were made to determine the effect of boron injury on the development of spot blotch and powdery mildew. The results are summarized in tables 3 and 4.

TABLE 4.—*The effect of applying solutions of sodium borate to the soil on the development of powdery mildew (Erysiphe graminis) and spot blotch (Helminthosporium sativum) when the plants were artificially inoculated in the greenhouse*

Variety	Stage of host development	Amount of sodium borate per pot	Degree of infection	
			Powdery mildew	Spot blotch
Black Hull-less, Selection	Boot	.1 gm.	M <sup>a</sup>	H
“ “	Boot	none	L +	M
Lion, C. I. 923 .....	Jointing	.1 gm.	M +	H
“ “ .....	“	none	H	H
Minsturdi, Minn. 437 .....	“	.1 gm.	T -	M -
“ “ .....	“	none	L +	L +
“ “ .....	Headed	.2 gm.	T -	M -
“ “ .....	“	none	L +	M +

<sup>a</sup> H = heavy infection; M = moderate; L = light; T = trace.

The presence of boron in sufficient quantities to cause considerable spotting did not seem to increase susceptibility to spot blotch nor to render the plants immune from or even resistant to the attack of powdery mildew. In these studies, when barley plants with considerable boron injury were inoculated with mildew and placed in a favorable environment, heavy infection always developed on the healthy green portions of the leaves (Fig. 6).

An attempt was made to determine the relative tolerance of *Helminthosporium sativum* and *Erysiphe graminis* spores to boron. *H. sativum* spores germinated normally in solutions containing 1 part of boric acid or borax in 500 parts of water plus host tissue. Although a fairly high percentage of *E. graminis* spores began to germinate in this concentration, most of their germ tubes did not develop normally. At lower concentrations both species germinated normally. Thus it would appear that *H. sativum* spores have a greater tolerance to boron than mildew. Conidia of both species germinated normally in distilled water to which bits of “boron-diseased” tissue of barley were added. Even if one assumes that there is practically no boron present in the necrotic spots, powdery mildew could



FIG. 6. Glabron Minn. 445 barley with considerable boron injury and heavy infection of powdery mildew. Note perithecial formation by the mildew.

not develop normally on the lesions, since it is an obligate parasite. On the other hand, *H. sativum* is a facultative parasite and grows very well on dead tissue. It is possible and also very likely that boron spots may serve as additional avenues of entrance for *Helminthosporium* spp.; but evidence obtained indicates that normal leaf tissues of boron-diseased plants are no more susceptible than those of uninjured plants.

#### CONTROL

Obviously, the leaf spots, if of nonparasitic origin, can not be controlled by seed treatment. One lot of seed from 4 varieties of barley was treated

with Ceresan, an organic mercury dust, at the rate of 3 oz. per bushel and another lot of the same varieties was treated by the Jensen modified hot-water method. Plants grown in the greenhouse from the seed developed as severe leaf spotting as those from nontreated seed.

It has already been pointed out that lime, copper, magnesium, and other compounds, when added to soil, failed to control the disease. It has been shown that certain nutritional diseases can be controlled by direct application of certain chemicals to the foliage (1). The writer also attempted to control the nonparasitic disease of barley by dusting and spraying. During the summer of 1932, 16 varieties of barley, 12 of which usually develop considerable leaf spot and 4 of which usually remain free from such disorders, were sown in 4 adjoining plots. One plot was sprayed with iron chloride 1:1000, and one with manganese sulphate 1:1000 on 4 different occasions about 7 to 10 days apart; the 3rd plot received several heavy applications of Kolo sulphur dust, and the 4th remained untreated. In no case did the treatment control, reduce, or intensify the disease. For example, the varieties Gorak C. I. 1086 and *Hordeum d. decorticutum* C. I. 2230 became heavily infected on all 4 plots, while 2 varieties, *H. v. duplinigrum* C. I. 2206 and Algerian C. I. 1179 remained essentially free on the 4 plots. The results were equally consistent for the 16 varieties tested.

The data in the first 3 columns in table 2 indicate that there are striking differences in varietal behavior to the physiological disease. Thus Algerian and *H. v. duplinigrum* remained free from spots, while Gorak and other varieties developed considerable infection.

#### DISCUSSION

It is evident that there are many nonparasitic leaf spots of barley. The symptoms are manifold and variable, depending on the cause of the spots and the environmental conditions under which they develop. The symptoms often are very similar to those caused by *Helminthosporium sativum* and other pathogens and can easily be confused with them. It has been shown definitely, however, that many of the spots are not caused by pathogens but by unfavorable soil conditions and inherent characters. In attempts to discover the soil factors responsible for the development of nonparasitic spots, boron was found to cause the development of spots quite similar to those caused by *H. sativum* and often indistinguishable from them. It seems very likely, therefore, that there has been confusion in the past with respect to the nature of many of the spots on barley. It is possible, also, that studies on varietal resistance have yielded questionable results because of confusion with respect to the cause of the spots. For example, the writer found that boron did not increase the susceptibility of

barley to *H. sativum* nor decrease its susceptibility to powdery mildew, as had previously been reported. This leads to the opinion that what has been considered spot blotch in the past was, in some instances, not spot blotch. It is apparent that the nature of parasitic and nonparasitic spots should be determined, for each variety used, in genetic studies and in tests of varietal resistance. It is possible that in many cases the existence of a large amount of nonparasitic spotting may serve as an indicator of the suitability of the soil for barley culture.

#### SUMMARY

In Minnesota, many different types of nonparasitic leaf spots are common on barley. The size, shape, color, and number and distribution of spots are variable. Many of the lesions are characteristic for a particular variety. Some of the lesions are similar to those produced by certain species of *Helminthosporium* and other fungi.

No evidence could be obtained that these spots were caused by a pathogen. No causal organisms were isolated. All evidence indicates that certain types of lesions are inherited, while others are the result of possible malnutrition or the presence of some toxic principle in the soil. Attempts to produce leaf spots with 20 different compounds were unsuccessful except with compounds containing boron.

It was demonstrated that boron, supplied either as boric acid or sodium borate, caused lesions that resembled spot blotch caused by *Helminthosporium sativum*.

Leaves of barley injured by boron were not rendered more susceptible to *Helminthosporium sativum* nor more resistant to *Erysiphe graminis* than nontreated plants. Less powdery mildew usually develops on boron-injured plants, chiefly because of the decreased area of healthy leaf tissue and because boron often causes premature killing of leaves and, consequently, less dense foliage.

The nonparasitic leaf spots could not be controlled by seed or soil treatment. Spraying with manganese sulphate and iron chloride and dusting with Kolo dust also were ineffective control measures.

Observations were made on severity of spotting on more than 125 varieties of barley. There were striking differences in their behavior. Some varieties always became severely spotted, others remained essentially free, while still others were intermediate in their behavior.

In some varieties the spots appear in the seedling stages, in others not until the boot or heading stage is reached.

Observations and tests in field and greenhouse over a period of years indicate that varietal behavior is relatively stable.

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# ISOLATION AND STUDY OF SOME YELLOW STRAINS OF CUCUMBER MOSAIC

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## INTRODUCTION

McKinney (7, 8) observed that tobacco plants infected with tobacco-mosaic virus frequently develop bright yellow spots in addition to the usual mottling symptoms. By cutting out the yellow spots and inoculating from them to healthy plants, he was able to isolate a virus that produced yellow-mosaic symptoms in tobacco. He suggested the possibility that yellow strains might arise from tobacco-mosaic virus by a process of mutation, using the term in its broadest sense. Jensen (4) also reported the occurrence of bright yellow spots in plants infected with tobacco-mosaic virus. He showed that not only one but a number of different yellow-mosaic viruses can be isolated from such spots. Jensen has shown further that these spots occur in plants infected with tobacco-mosaic virus that was separated from other viruses by successive passages through local lesions in *Nicotiana glutinosa* L.

Tobacco (*Nicotiana tabacum* L. var. Turkish) plants, infected with cucumber-mosaic virus, frequently develop bright yellow spots similar to those described for tobacco mosaic by McKinney and Jensen. From such spots, it has been possible to isolate several different viruses that produce yellow mosaic or necrotic symptoms in tobacco. Evidence has been obtained to show that these viruses are not the result of accidental infection but arise in infected plants by mutation or a similar process. The purpose of the present paper is to describe the isolation of and symptoms produced by these viruses and to present the evidence obtained in regard to their origin.

## MATERIALS AND METHODS

Except where otherwise mentioned, a cucumber-mosaic virus obtained from Dr. R. H. Porter was used. The virus was designated by him (9) as "white pickle mosaic virus" or "cucumber virus 1." It is referred to in this paper as Porter's cucumber-mosaic virus. Several experiments were conducted with a strain of virus obtained from Dr. E. M. Johnson and classified by him (5) as "cucumber mosaic type 1 virus." When this virus is mentioned it will be referred to as Johnson's cucumber-mosaic virus. The two viruses are similar, but comparative inoculations to Turkish tobacco and other hosts have shown them to be different from each other.

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FIG. 1. A bright yellow spot in a leaf of a Turkish tobacco plant infected with Porter's cucumber-mosaic virus.

The symptoms produced by them in several hosts are described in a later section of the paper.

Reasonable care was taken to avoid accidental infection. Plants were grown in 4-inch pots in a greenhouse that was fumigated frequently to control insects. Inoculations were made by means of the rubbing method, except in a few instances in which the pin-puncture method was employed. Instruments used in making inoculations were sterilized by autoclaving or by boiling for a few minutes.

#### ISOLATION OF VIRUSES FROM YELLOW SPOTS

Bright yellow spots have been observed frequently in tobacco plants having cucumber-mosaic disease. They vary in size, shape, and intensity of the yellow color. They may develop within a few days after the appearance of the systemic disease, or they may not develop until plants have been infected for a month or more. Figure 1 shows a bright yellow spot that occurred in a tobacco plant infected with Porter's cucumber-mosaic virus. The conditions favoring frequent development of such spots have not been determined. The spots occur more frequently in plants making rapid growth than in slow-growing plants.

Inoculations were made from a number of bright yellow spots in order to determine whether viruses differing from Porter's cucumber mosaic could be isolated from them. Discs of tissue, each containing a single spot, were cut out with a sterile cork borer, macerated on a glass slide with a glass spatula, and rubbed over leaves of healthy tobacco plants. The glass spatulas used for making inoculations were similar to those described by Samuel (11). In 3 or 4 days, yellow local lesions developed in the inoculated leaves. Pin-puncture inoculations were made from the local lesions to healthy tobacco seedlings in flats. Out of a total of 956 single pin-puncture inoculations, infection was obtained from 14. In all cases, the first appearance of infection was the development of a chlorotic or necrotic area around the pin puncture. The infection gradually spread from these areas to other parts of the leaf and eventually involved younger leaves of the seedlings. Transfers were made from infected seedlings to healthy tobacco plants in pots. Five of the viruses thus isolated were selected for further study in order to determine the ways in which they differ from each other. A comparison was made of the symptoms produced by them in *Cucumis sativus* L. var. Davis Perfect, *Lycopersicon esculentum* Mill. var. Bonny Best, *Spinacia oleracea* L. var. Henderson's Long Season, *Nicotiana glutinosa* L., *N. langsdorffii* Schrank., and *N. tabacum* L. var. Turkish. Each of the viruses was tested by inoculation of 3 plants of each host. The results were confirmed by inoculation of one or more additional sets of 3 plants each. Controls were provided by rubbing an equal number of sim-

ilar plants with healthy juice. On the basis of the symptoms produced, all 5 of the viruses studied proved to be different from each other. They appear to be closely related to cucumber-mosaic virus and are considered to be strains of the latter.

#### SYMPTOMS PRODUCED BY VIRUSES ISOLATED FROM YELLOW SPOTS

In the following paragraphs, a description is given of the symptoms produced by 8 different strains of cucumber-mosaic virus. ✓ These strains include Porter's cucumber-mosaic virus, Johnson's cucumber-mosaic virus, 5 strains that were obtained by inoculation from yellow spots produced by Porter's cucumber-mosaic virus, and an additional strain of yellow mosaic (strain 6) that was isolated by inoculation from a plant infected with strain 5. All 8 strains have been passed in serial transfers through tobacco plants. With the exception of strain 5, they have produced symptoms that are constant.

*Porter's Cucumber-mosaic Virus.* The virus has been studied for a period of more than a year. It produces yellow primary lesions in *Nicotiana langsdorffii* and spinach, and, under certain conditions, inconspicuous yellow primary lesions in Turkish tobacco. The clearing-of-veins symptom is produced in cucumber, tomato, *N. glutinosa*, *N. langsdorffii*, and tobacco. The virus produces a coarse, green and light green mottling in all 5 of these hosts. The mottling in tobacco is illustrated in figure 2, A. Affected plants also show leaf distortion, blistering, crinkling, and stunting. In *N. glutinosa*, *N. langsdorffii*, and tobacco, the distortion and stunting are severe. These symptoms are usually less severe in cucumber and tomato. *N. glutinosa* and tobacco plants frequently show temporary recovery from the effects of the disease and develop several healthy-appearing leaves. In such instances, the next group of leaves to develop shows the typical mottling symptoms.

*Johnson's Cucumber-mosaic Virus.* This virus is characterized by the production of symptoms, similar, in general, to those resulting from infection with Porter's cucumber-mosaic virus. However, the two viruses may be readily differentiated by their reactions in certain hosts. In *Nicotiana glutinosa*, *N. langsdorffii*, spinach, tomato, and Turkish tobacco, the symptoms of the virus of Johnson's cucumber mosaic are milder than those of Porter's cucumber mosaic. In all 5 hosts, the former virus causes considerably less stunting and distortion. Tobacco plants that have been infected for periods of two weeks or more sometimes develop in their lower leaves a faint grayish necrosis that tends to follow small veins. This necrosis and the mottling that accompanies it are illustrated in figure 2, B.

*Strain 1.* This virus produces symptoms similar to those of the two viruses described above but that differ from them in certain characteristic respects. In all the hosts tested, with the possible exception of spinach,

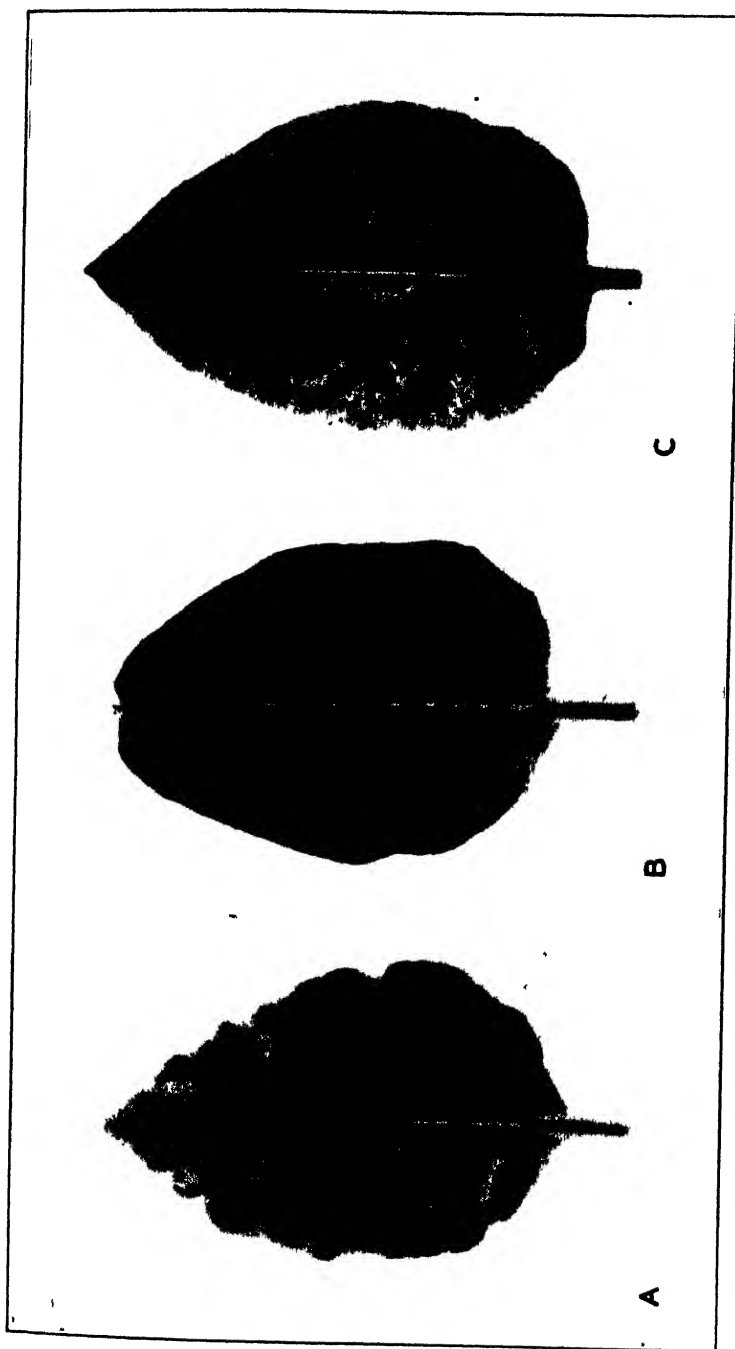


FIG. 2. Typical leaves from Turkish tobacco plants infected with cucumber-mosaic virus. A. Porter's cucumber mosaic. B. Johnson's cucumber mosaic. C. Strain 1.

it produces the least stunting and distortion of any of the cucumber-mosaic viruses so far studied. In *Nicotiana glutinosa*, *N. langsdorffii*, and Turkish tobacco, it produces grayish, necrotic primary lesions. In systemically diseased leaves of these hosts, it causes a yellow, blotch-like mottling frequently associated with yellow, zonate rings. In *N. glutinosa*, the yellowing tends to follow the larger veins. In tobacco, the yellowing appears in patches between the larger veins, as shown in figure 2, C. The yellowing in most of the hosts studied is more intense than that produced by either of the viruses described above but much less intense than that produced by some of the other strains that were isolated. In tomato, the virus causes only a mild mottling with almost no stunting or leaf distortion.

*Strain 2.* The symptoms produced by virus of strain 2 are similar to those of Porter's cucumber-mosaic virus but are much more brilliant. Bright yellow primary lesions are produced in spinach, tomato, *Nicotiana glutinosa*, *N. langsdorffii*, and Turkish tobacco. In the last 4 of these hosts, strain 2 causes a bright yellow or white, blotch-like, mottling and spotting. Figure 3, A, shows the type of mottling produced in tobacco. In cucumber, the mottling is more brilliant than that resulting from infection with the previously described viruses.

*Strain 3.* The symptoms produced by this virus in most of the hosts studied appear to be identical with those of strain 2. However, the two viruses may be differentiated by the symptoms they produce in *Nicotiana langsdorffii* and tomato. The virus of strain 3 moves a little more rapidly and produces less severe symptoms in *N. langsdorffii* and tomato than virus of strain 2. Tomato plants are not stunted quite so severely by strain 3 as they are by strain 2.

*Strain 4.* This virus, although apparently closely related to strains 2 and 3, produces less severe symptoms in some hosts. Usually the mottling is less brilliant and the stunting less severe in tomato, *Nicotiana glutinosa*, *N. langsdorffii*, and tobacco than that resulting from infection with either strain 2 or strain 3. Strain 4 produces a yellow or white mottling and spotting in all 4 of these host plants.

*Strain 5.* This virus produces symptoms strikingly different from those resulting from infection with the other strains studied. It causes necrotic primary lesions in tomato, *Nicotiana glutinosa*, *N. langsdorffii*, Turkish tobacco, and spinach, and yellow and necrotic primary lesions in cucumber. The first 4 of these hosts develop a systemic necrosis when infected. The necrosis is usually present as solid spots or zonate rings, but may occasionally follow veins and produce oak-leaf patterns. Figure 3, B, shows the symptoms produced by this strain in tobacco.

Strain 5 does not appear to be stable. In systemically diseased leaves of several of the hosts studied, it invariably gives rise to yellow-mosaic

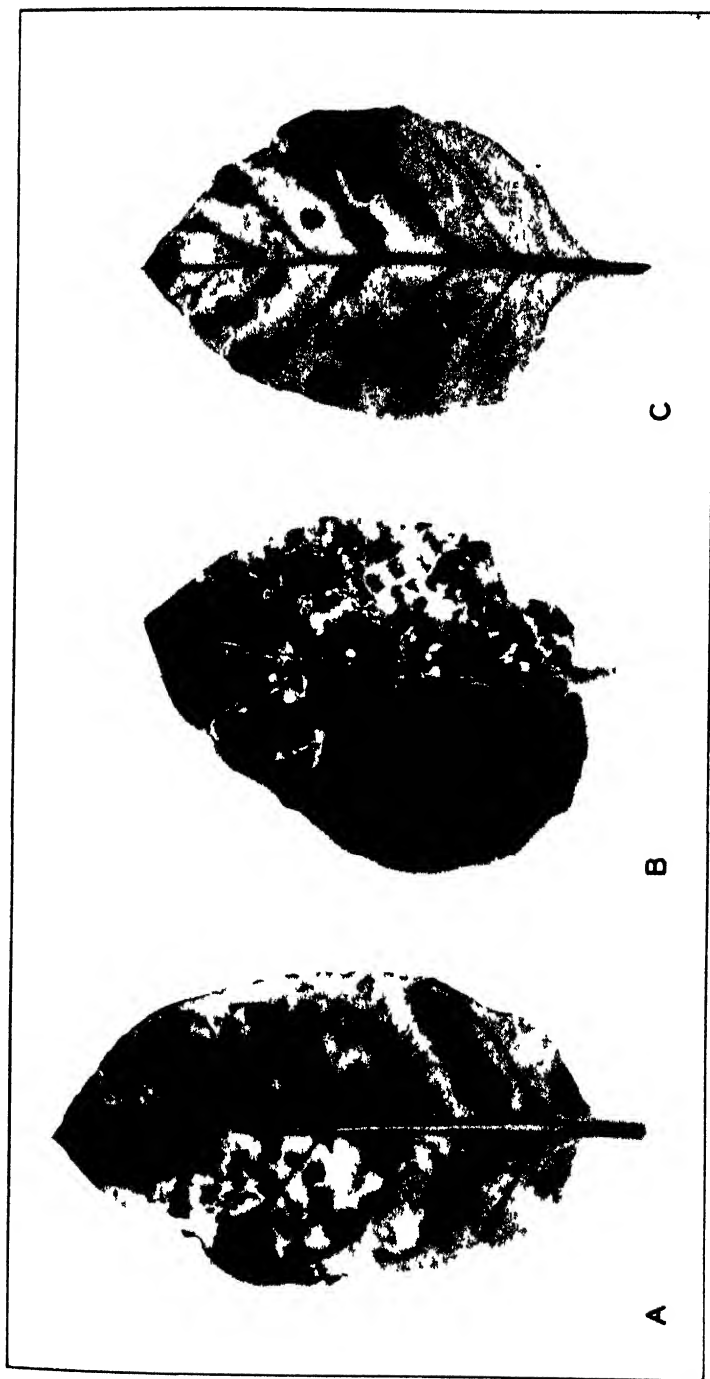


FIG. 3 Typical leaves from Turkish tobacco plants infected with cucumber mosaic virus. A. Strain 2. B Strain 5 C. Strain 6

symptoms. By inoculating from leaves showing yellow-mosaic symptoms, a yellow-mosaic virus and a green-mosaic virus have been obtained free from the virus of strain 5. A description of one of these viruses (strain 6) is given below. The question of the origin of the yellow- and the green-mosaic viruses is considered in another section of the paper.

✓ *Strain 6.* This virus was obtained by inoculation from the tip of a tobacco plant infected with strain 5. It produces the most brilliant symptoms of any of the viruses described in this paper. It causes yellow primary lesions in Turkish tobacco, cucumber, spinach, tomato, *Nicotiana glutinosa*, and *N. langsdorffi*. Systemic symptoms in all these plants consist of a mottling of brilliant yellow and dark green. Infected leaves of tobacco, *N. glutinosa*, and spinach occasionally become almost entirely yellow. More frequently, the yellowing is confined to certain portions of the leaves, the remaining portions being green. Figure 3, C, shows the symptoms of strain 6 in tobacco.

#### PROPERTIES OF THE VIRUSES OBTAINED FROM YELLOW SPOTS

The evidence reported in preceding paragraphs indicates that the viruses under discussion, although closely related, differ in the property (or properties) responsible for the expression of a symptom complex in the host plant. It is immediately of interest to know whether these viruses differ significantly in other properties. For this reason, tests were made to determine the thermal death points (for a 10-minute exposure) of the strains described above and their ability to withstand aging *in vitro*. The method used for heating virus samples was the same as that employed (10) in studies on the thermal death rate of tobacco-mosaic virus. For determining ability to withstand aging *in vitro*, the virus samples were kept in tightly stoppered 1 x 10 cm. test tubes in an oven held at 29° C. All tests were made with freshly extracted juice of mosaic-diseased tobacco plants. Infectivity of the virus samples was determined by inoculation of 10 young Turkish tobacco plants.

Results of tests on thermal death point are presented in table 1; those on aging *in vitro* are given in table 2. The tables show that there is little or no difference between the strains of cucumber-mosaic virus in thermal death point or ability to withstand aging *in vitro*. Since only a single test was made for each virus, the differences that were obtained may be the result of normal variation rather than the result of differences in reaction of individual viruses. It is possible, of course, that a more thorough study may reveal slight, but constant, differences. It is concluded that the viruses are similar in so far as their resistance to heating and to aging *in vitro* is concerned.



TABLE 1.—*Effect of exposure of different strains of cucumber-mosaic virus to various temperatures for 10 minutes*

Temperature in degrees C.	Porter's cucumber mosaic	Johnson's cucumber mosaic	Strain No. 1	Strain No. 2	Strain No. 3	Strain No. 4	Strain No. 5	Strain No. 6
60 .....	3 <sup>a</sup>	4	1	10	2	1	5	4
64 .....	5	2	0	9	0	2	1	1
66 .....	2	0	0	6	1	1	1	0
68 .....	4	2	0	2	0	0	1	0
70 .....	2	0	0	0	0	0	2	0
72 .....	2	0	0	0	0	0	0	0
74 .....	0	0	0	0	0	0	0	0
76 .....	0	0	0	0	0	0	0	0
80 .....	0	0	0	0	0	0	0	0
Unheated controls .....	10	10	10	10	10	10	10	10
Healthy controls .....	0	0	0	0	0	0	0	0

<sup>a</sup> Figures represent the number of Turkish tobacco plants infected of 10 inoculated with the virus sample indicated.

TABLE 2.—*Effect of exposure of cucumber-mosaic virus at 29° C. for certain periods of time*

Time of exposure	Porter's cucumber mosaic	Johnson's cucumber mosaic	Strain No. 1	Strain No. 2	Strain No. 3	Strain No. 4	Strain No. 5	Strain No. 6
0 days .....	10 <sup>a</sup>	10	10	10	10	10	10	10
1 day .....	9	10	3	10	0	8	4	3
2 days .....	5	10	2	0	4	5	3	0
3 " .....	4	10	0	1	0	0	0	0
4 " .....	0	1	0	2	0	0	0	0
5 " .....	0	0	0	0	0	0	0	0

<sup>a</sup> Figures represent the number of Turkish tobacco plants infected of 10 inoculated with the virus sample indicated. An equal number of controls, inoculated with healthy juice treated identically as diseased juice, remained healthy.

#### ORIGIN OF VIRUSES PRESENT IN YELLOW SPOTS

It having been shown that a number of different viruses can be isolated from yellow spots in tobacco plants infected with cucumber-mosaic virus, attention was directed to the question of the origin of such viruses. The frequent occurrence of yellow spots in mosaic-diseased plants, together with the failure to observe such spots in any of the healthy controls, proves that the viruses present in the yellow spots are not ordinarily the result of acci-

dental infection. Experiments were undertaken to determine whether such viruses were present in the original inoculum or had some other origin. An attempt was made to secure pure lines of cucumber-mosaic virus for use in these experiments. While there are several different methods for separation of closely related viruses, the primary-lesion method appears to be the most satisfactory for this purpose. This method was used in the present investigation.

✓ In preliminary tests, it was found that leaves of several varieties of cowpea, *Vigna sinensis* Endl., would develop necrotic primary lesions when rubbed with cucumber-mosaic virus. Table 3 shows the results of these tests. The lesions appear within 2 days after inoculation and consist of circular, dark red, necrotic spots. They vary in size but usually are between 1 and 2 millimeters in diameter. Figure 4 shows the type of lesion that is produced in Black Eye cowpeas. The virus of cucumber mosaic is recoverable by subinoculation from the local lesions it produces in cowpeas.

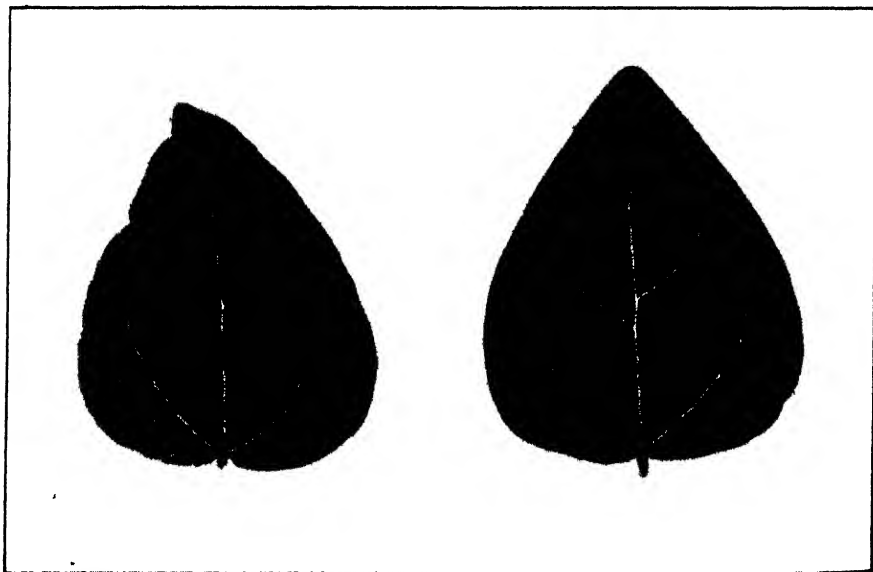


FIG. 4. Necrotic primary lesions produced in Black Eye cowpea by inoculation with Porter's cucumber-mosaic virus. A healthy leaf is shown for comparison.

For the purpose of securing a pure line of virus, Porter's cucumber-mosaic virus was passed through successive transfers in primary lesions. Cowpea (var. Black Eye) plants were used in the series of transfers because the local lesions produced in this host, being necrotic, do not allow extensive spread of virus. After 10 successive transfers had been made

TABLE 3.—*Effect of inoculating varieties of cowpeas with cucumber-mosaic virus*

Variety	No. plants inoculated with juice of diseased plants	No. plants developing lesions	Total lesions	No. plants inoculated with juice from healthy plants (Controls) <sup>a</sup>
Black .....	72	46	965	67
Black Eye .....	63	40	184	21
Brabham .....	23	2	5	5
Clay .....	140	62	398	104
Early Buff .....	24	4	5	8
Groit .....	22	1	1	4
Iron .....	120	19	31	104
New Era .....	108	41	74	95

<sup>a</sup> Lesions did not develop in any of the controls inoculated by rubbing with juice of healthy plants.

and a pure line of virus was believed to have been established, the disease was transferred to tobacco plants for the purpose of determining whether or not yellow spots would be produced. Local lesions obtained on the 10th transfer were cut out with a sterile cork borer and used to inoculate healthy tobacco plants. Thirty-six tobacco plants were inoculated, a single lesion serving as inoculum for each plant. An equal number of controls were provided by rubbing leaves of tobacco plants with juice extracted from healthy-appearing tissues of the same cowpea leaves. Of the 36 plants inoculated with virus from local lesions, 25 became infected and developed symptoms typical of Porter's cucumber mosaic. A careful examination was made of the plants 3 weeks after inoculation. It was found that bright yellow spots had developed in 17 of them. Five of the plants showed 1 spot each, nine showed 2 spots each, two showed 3 spots, and one showed 4 spots. Eight of the infected plants had not developed yellow spots at the time the observation was made. All the controls were healthy and free from yellow spots. To determine whether or not yellow-mosaic viruses were present in the yellow spots, inoculations were made from 10 of the spots to healthy tobacco plants. All 10 of the inoculated plants developed yellow-mosaic and green-mosaic symptoms. It is concluded that yellow-mosaic viruses occur in tobacco plants infected with a green-mosaic virus previously passed through 10 successive transfers in primary lesions.

The experiment was repeated with Johnson's cucumber-mosaic virus after it had been subjected to 10 successive passages through cowpea local lesions. A number of the plants infected with this cucumber mosaic showed bright yellow spots. None of the controls showed yellow spots.

It is believed that a pure line was obtained by passing cucumber-mosaic virus through 10 successive transfers in primary lesions. It might be argued, however, that several strains of the virus were carried simultaneously in the same local lesions. In order to meet this objection, an attempt was made to separate two closely related viruses by passing them through local lesions in cowpea. Subinoculations were made to tobacco plants from single necrotic lesions produced in cowpeas by inoculation with a mixture of equal parts of a green (Porter's cucumber-mosaic virus) and a yellow (strain 2) cucumber-mosaic virus. Infection was obtained in 39 of the tobacco plants; 16 showed symptoms of green mosaic, 22 showed symptoms of yellow mosaic, and 1 developed symptoms of a mixture of green and yellow mosaics. From the tobacco plants that developed symptoms characteristic of infection with the green mosaic, only the green-mosaic virus was recoverable by inoculation to cowpea and subinoculation from single necrotic lesions. In the same way, only the yellow-mosaic virus was recoverable from the tobacco plants that developed symptoms characteristic of infection with yellow mosaic. From a single plant showing symptoms of a mixed infection, both viruses were recoverable by subinoculation. The results indicate that, except in a small percentage of the cases tested, the green-mosaic and the yellow-mosaic viruses were separated completely by passing them through necrotic local lesions in cowpea. It is realized that these results are not conclusive. It may be that the yellow-mosaic virus cannot be separated completely from the green. Nevertheless, the experiments add more weight to the conclusion that the strains of virus isolated from plants infected with a green-mosaic virus passed through 10 successive transfers in cowpea, were not present in the original inoculum but must have had some other origin. It is believed that yellow-mosaic viruses arise in plants infected with a green cucumber-mosaic virus.

#### ORIGIN OF VIRUSES ISOLATED FROM STRAIN 5

The virus of strain 5 produces necrotic lesions in Turkish tobacco. It is believed that the virus may be separated completely from other viruses with which it is mixed by successive inoculations from such necrotic lesions. When tobacco plants were inoculated with virus of strain 5, freed from other viruses by inoculations from a series of necrotic lesions, they invariably developed symptoms of yellow mosaic in addition to the usual necrotic symptoms. From such plants, a yellow-mosaic virus and a green-

mosaic virus were isolated. Evidence has been obtained to show that these viruses were not present in a mixture with strain 5, but that they arose in the plants infected with this strain. In a set of 83 tobacco plants infected with virus of strain 5, every plant developed local and systemic necrotic symptoms. All of the infected plants also developed yellow-mosaic symptoms eventually. Twenty-four showed yellow-mosaic symptoms on the 11th day after inoculation, 28 showed yellow-mosaic symptoms on the 14th day, 6 showed yellow-mosaic symptoms on the 16th day, 6 showed yellow-mosaic symptoms on the 22nd day, while 19 did not show yellow-mosaic symptoms until a month or more after inoculation. It is believed that if the yellow-mosaic viruses had been present in the original inoculum, all the infected plants would have shown yellow-mosaic symptoms at about the same time. Evidence for this belief was obtained in the following experiment. Ten tobacco plants were inoculated with virus of strain 5, 10 were inoculated with a sample of the same virus to which had been added virus of strain 6 in the proportion of 1 part of strain 6 to 9 parts of strain 5, while 10 similar plants were rubbed with water and held as controls. All of the 20 plants that were inoculated with strain 5 developed necrotic local lesions. The 10 plants that were inoculated with the mixture developed both yellow and necrotic local lesions. All 10 plants inoculated with the mixture developed systemic symptoms of yellow mosaic on the 7th day after inoculation. Those infected with strain 5 developed systemic symptoms on the 9th day after inoculation, but none of them showed symptoms of yellow mosaic until the 18th day. Most of them required an even longer time to show yellow-mosaic symptoms. It is concluded that both the yellow-mosaic and the green-mosaic viruses arose in plants infected with strain 5. Strain 5 appears to be more unstable than the other viruses isolated from bright yellow spots.

#### ALTERATION OF CUCUMBER-MOSAIC VIRUS IN PRIMARY LESIONS

In an experiment in which Johnson's cucumber-mosaic virus was passed from primary lesions in successive transfers, evidence was obtained that the virus may occasionally become altered in a primary lesion. Since this phenomenon is of considerable interest in connection with the origin of yellow-mosaic viruses and has not been reported previously, the evidence will be given here.

The virus of Johnson's cucumber mosaic was rubbed over leaves of several cowpea plants. One of the lesions produced was cut out and used to inoculate other cowpea plants. This inoculation may be spoken of as the first transfer. Controls were provided in the first and subsequent transfers by inoculation of an equal number of cowpea leaves with juice of a healthy cowpea plant. A pure line of virus was established by making

inoculations from a single, isolated lesion on the 2nd, 3rd, 4th, 5th, and 6th transfers. This line is represented by the letter G in table 4. On the 7th transfer, 3 related lines were established by inoculating from 3 single lesions of the 6th transfer. These are represented by G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> in table 4. On the 9th transfer, an additional pure line was established by making inoculations from 2 necrotic lesions produced by line G<sub>2</sub> on the 8th transfer. Leaves inoculated with virus from one of these lesions developed both yellow and necrotic primary lesions. Subsequent transfers, referred to as Y<sub>1</sub> in table 4, were made only from the yellow lesions. On the 10th transfer, line G<sub>3</sub> developed both yellow and necrotic lesions. Subsequent transfers from this line, designated as Y<sub>2</sub>, were likewise made only from the yellow lesions. Virus from the yellow primary lesions of lines Y<sub>1</sub> and Y<sub>2</sub> produced yellow lesions when inoculated into cowpea. The remaining 2 lines, G<sub>1</sub> and G<sub>2</sub>, continued to yield only necrotic lesions in subsequent transfers. Cowpeas in which yellow lesions occurred developed symptoms that involved the young leaves and stem and eventually caused the death of affected plants. When transmitted to tobacco, on the 13th transfer, the virus present in the yellow lesions produced a yellow mosaic. Tobacco plants inoculated with virus from necrotic lesions of lines G<sub>1</sub> and G<sub>2</sub> developed green-mosaic symptoms.

TABLE 4.—Serial transfers of Johnson's cucumber mosaic in cowpeas showing origin of strains Y<sub>1</sub> and Y<sub>2</sub>

Transfer No.													Symptoms produced in tobacco by inocu- lation from single lesions of the 13th transfer	
1	2	3	4	5	6	7	8	9	10	11	12	13		
G → G → G → G → G →						↗							G <sub>1</sub> → G <sub>1</sub> → G <sub>1</sub> → G <sub>1</sub> → G <sub>1</sub> → G <sub>1</sub> → G <sub>1</sub>	Green mosaic
						↘							G <sub>2</sub> → G <sub>2</sub> → G <sub>2</sub> → G <sub>2</sub> → G <sub>2</sub> → G <sub>2</sub>	Green mosaic
						↘							G <sub>2</sub> <sup>a</sup>	Yellow mosaic
													Y <sub>1</sub> → Y <sub>1</sub> → Y <sub>1</sub> → Y <sub>1</sub> → Y <sub>1</sub> → Y <sub>1</sub>	
						↘							G <sub>3</sub> <sup>a</sup>	Yellow mosaic
													G <sub>3</sub> → G <sub>3</sub> → G <sub>3</sub> → Y <sub>2</sub> → Y <sub>2</sub> → Y <sub>2</sub> → Y <sub>2</sub>	

<sup>a</sup> Transfer in which yellow primary lesions first appeared. Subsequent transfers were made only from the yellow lesions in these two lines.

On the 13th transfer, the virus of line Y<sub>1</sub> and Y<sub>2</sub> was inoculated into additional sets of cowpea plants. Yellow primary lesions were produced. In many cases, but not all, a necrotic ring partially or completely en-

circled the lesion. When the disease became systemic, affected plants were not killed as in previous instances but developed a green and yellow mottling in the newly formed leaves. The disease is illustrated in figures 5 and 6. Virus from the mottled leaves has produced in all cases up to the present time yellow primary lesions, with or without a necrotic periphery, followed by typical mosaic mottling in systemically affected leaves. It is not yet known whether the virus was again altered by further passage through cowpea or whether the conditions of the experiment modified the course of the systemic infection.

The yellow-mosaic virus of lines  $Y_1$  and  $Y_2$  differs from all the other yellow-mosaic viruses previously referred to (strains 1, 2, 3, 4, 5, and 6), since none of the others produce yellow primary lesions nor become systemic in cowpea plants to which they are transferred. This strain of cucumber-mosaic virus produces a severe mosaic disease in cowpeas.

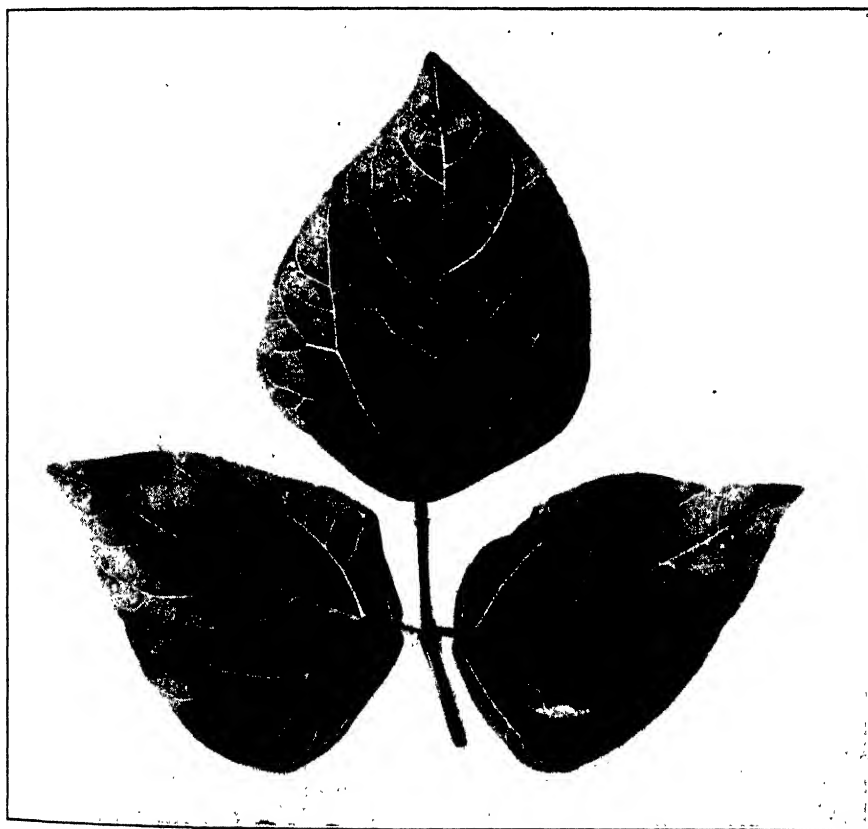


FIG. 5. Mottled leaf of Black Eye cowpea infected with cucumber-mosaic virus, strain  $Y_1$ .

## DISCUSSION

The results obtained in this investigation have established the fact that several different but closely related yellow-mosaic viruses can be isolated from bright yellow spots produced in tobacco plants by inoculation with ordinary green cucumber-mosaic virus. It has been shown further that these viruses are not the result of accidental infection and that they may occur in plants infected with a green-mosaic virus that previously was passed through a series of primary necrotic lesions. While it can be argued that a mixture of several cucumber-mosaic viruses might be carried simultaneously through serial transfers in local lesions, evidence has been obtained to show that this is not the case. It was found that 2 closely related cucumber-mosaic viruses may be separated completely, so far as could be determined with the experimental methods available, by passing them



FIG. 6. Black Eye cowpea plants infected with cucumber-mosaic virus, strain Y., showing severe stunting, mottling, and distortion. Healthy controls on the right are presented for comparison.

through local lesions in cowpea. Similar results were obtained with tobacco-mosaic virus by Jensen (4) and by Kunkel (6). Jensen (4) presented evidence to show that 2 closely related tobacco-mosaic viruses could be separated by inoculation from single local lesions produced in *Nicotiana glutinosa* with a mixture of the 2 viruses. Kunkel (6) showed that from single necrotic lesions produced in *N. langsdorffii* by inoculation with mixtures of tobacco- and aucuba-mosaic viruses, one or the other of the viruses, but seldom both, is recoverable by subinoculations to *N. sylvestris*.



On first thought, it might seem that the sudden appearance of the yellow-mosaic viruses,  $Y_1$  and  $Y_2$ , in cowpeas after 8 successive passages of a green-mosaic virus constitutes evidence that the primary lesion method is not suited for separation of closely related strains of virus. It is recognized as a possibility that these viruses might have been present as a mixture in the necrotic lesions with the green-mosaic virus and that separation was not accomplished until after 8 successive transfers had been made. On the other hand, it seems probable that if the yellow-mosaic virus had been present in the first passage it would have become systemic in the cowpea plants to which it was transferred. It appears, therefore, that the explanation which best fits the facts in the case is that only the green-mosaic virus was present in the first 7 transfers and that it suddenly became altered and gave rise to a yellow-mosaic virus. It is difficult to avoid the conclusion that yellow-mosaic viruses arise in plants infected with cucumber-mosaic virus. Such viruses arise in primary lesions, as well as in systemically diseased leaves.

The exact manner in which yellow-mosaic viruses are produced has not been determined. Assuming a chemical nature for the virus, it may be that new strains arise by a rearrangement or isomerization of a group or groups in the virus unit. A second possible explanation for the origin of yellow-mosaic viruses is that they are derived from a green-mosaic virus by a process similar, in general, to mutation in living organisms. It is left for future work to show whether this change in the virus results from a sudden alteration in a particular virus unit and subsequent multiplication of that unit, whether the virus in a certain cell of the plant host stimulates the production by that cell of another strain of virus, or whether there is some entirely different explanation.

One of the strains of cucumber-mosaic viruses isolated from yellow spots was found to be unstable. From a necrotic-type virus (strain 5), it has been possible to isolate a yellow-mosaic and a green-mosaic virus. Evidence was obtained to show that neither the yellow-mosaic nor the green-mosaic virus was present in the original inoculum, but that both viruses arose in infected plants. The other strains isolated from yellow spots may also prove to be unstable on further study.

Whatever the origin of yellow-mosaic viruses, they are readily obtained from Porter's cucumber-mosaic virus and presumably from other field types of cucumber mosaic. It is probable that the development of strains of cucumber mosaic is much more common than one would be led to suppose from the frequency with which bright yellow spots occur in infected plants. Strains that do not produce such a marked effect in the tissues of the leaf may also be present. It is possible that a plant infected with a single strain of virus may eventually come to contain several green

or masked strains, as well as one or more yellow strains. The green and masked strains would be difficult to detect unless they were separated from other strains present in the same plant.

Many different strains of cucumber-mosaic virus are known to occur in nature. Jagger (3) distinguished between the ordinary "white-pickle" mosaic and a second mosaic that mottled the leaves of cucumber but showed no effect on fruit. Bewley (1) observed 2 types of cucumber mosaic in Great Britain. Johnson (5) distinguished 3 types of cucumber mosaic that occur in tobacco in Kentucky. Two additional types of cucumber mosaic were observed recently in Kentucky (2). Porter (9) described a cucumber mosaic differing from the ordinary mosaic and characterized by the development of yellow blotches. McKinney (8) describes yellow mosaics occurring in the cucurbits *Momordica charantia* L. and *Kedrostis foetidissima* Cogn. in West Africa. The conclusions arrived at here in regard to the origin of yellow- and green-mosaic viruses offer an explanation for the occurrence of the many different strains of cucumber-mosaic virus present in nature. The same phenomena may explain the occurrence of so many different virus diseases of plants. It should be pointed out, however, that, so far as they have been tested, the strains of cucumber mosaic that were isolated from yellow spots differ only slightly in their properties and appear to be closely related viruses. It has not yet been shown that one plant virus may give rise to a second virus differing as markedly in its properties from the first virus as, for instance, cucumber-mosaic and tobacco-mosaic viruses differ from each other.

On the other hand, it has been shown that a strain of cucumber-mosaic virus, producing only necrotic primary lesions in cowpea, may give rise to another strain that becomes systemic and produces a severe mosaic disease in this host. Whether this strain of cucumber mosaic is identical with the cowpea mosaic described by Smith (12) is not known. The results reported here suggest that new strains of viruses capable of attacking entirely new host plants may arise naturally in the manner described.

#### SUMMARY

Tobacco plants infected with cucumber-mosaic virus frequently develop bright yellow spots from which different but closely related yellow-mosaic and necrotic-type viruses may be isolated. When the virus of cucumber mosaic is passed in serial transfers through the necrotic primary lesions that it produces in cowpea, *Vigna sinensis* Endl., and is subsequently inoculated to tobacco, it continues to produce bright yellow spots in addition to the usual mottling symptoms. Evidence is thus obtained that strains of cucumber-mosaic virus arise by mutation or a similar process in tobacco plants having the cucumber-mosaic disease. Evidence also was obtained to

show that strains of cucumber-mosaic virus may arise in primary lesions, as well as in systemically diseased leaves of affected plants. One of the strains of virus isolated from bright yellow spots was found to be unstable and to yield additional strains on subinoculation. With one exception, the strains of cucumber-mosaic virus studied produce necrotic primary lesions in cowpea but do not become systemic in this host. However, a strain obtained by serial passages through such necrotic lesions causes a typical mosaic disease in cowpea.

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# INHERITANCE OF RESISTANCE TO BUNT AND LEAF RUST IN THE WHEAT CROSS, ORO $\times$ TENMARQ<sup>1</sup>

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## INTRODUCTION

Bunt of wheat, caused by *Tilletia levis* Kühn, is one of the most serious diseases of wheat in Kansas, where, according to Melchers (14), it caused a loss of about 15,000,000 dollars in 1926. Since it is difficult to impress the wheat grower with the importance of seed treatment as a method of bunt control, the most satisfactory solution would seem to be the use of resistant varieties. The problem of producing such varieties has also presented the opportunity to study the inheritance of resistance to bunt.

This paper deals primarily with the manner of inheritance of resistance to *Tilletia levis*, in the wheat cross, Oro  $\times$  Tenmarq. It also considers briefly resistance to leaf rust (*Puccinia triticina* Eriks.) in this cross.

## REVIEW OF LITERATURE

The production of bunt-resistant varieties of wheat was one of the successes of William Farrer (9), the well-known pioneer Australian plant breeder. Since that time, many crosses have been made in the United States and other countries in attempts to secure bunt-resistant varieties of wheat embodying those characters necessary to make them popular with both the farmer and the miller. In recent years investigators have studied wheat hybrids to determine as nearly as possible the mode of inheritance of resistance to bunt.

Gaines (10) concludes from a study of the resistance to bunt in Turkey  $\times$  Hybrid 128 and Turkey  $\times$  Florence that most of the factors for resistance are recessive. He considers that the inheritance of resistance to bunt is due to multiple factors and that all varieties of wheat do not possess the same factors for resistance.

Gaines and Singleton (12) and Aamodt (1) reported transgressive segregation in a number of F<sub>3</sub> lines. Briggs (3, 4, 5, 6, 7), in researches involving one physiologic form of the bunt organism, concludes that the inheritance of resistance to bunt is governed largely by 1 dominant factor, as in Martin, White Odessa, Banner Berkeley, and others, or 2 such factors, as in Hussar. He believes there may be other factors for resistance in these

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varieties, which would become evident if they were tested with other physiologic forms of the bunt organism (6). Bressman (2) found the deviation from the expected too great in the Hybrid 128  $\times$  Martin cross to explain the resistance of Martin to 3 collections of smut on a 1-factor basis. Churchward (8), working with Florence  $\times$  Hard Federation, obtained evidence that seemed to show the presence of a 1-factor difference for resistance to bunt in which susceptibility was dominant. Aamodt (1) and Kilduff (13), from a study of inheritance of resistance to bunt, obtained evidence indicating the presence of several factors governing reaction to bunt.

#### METHODS AND MATERIALS

The cross, Oro C.I. 8220  $\times$  Tenmarq Sel. Ks. No. 2637, was made by John H. Parker of the Kansas Agricultural Experiment Station, the purpose being to combine the desirable characters of Tenmarq, namely, excellent grain quality, high yielding capacity, and earliness, with the bunt resistance and winter hardiness of Oro.

#### PARENTAL VARIETIES

Oro is a pure-line selection made by H. M. Woolman in 1921 at the Sherman County Branch Station, Moro, Oregon, from a variety labeled Turkey No. 889, received from the United States Department of Agriculture. Tests at the Moro Station showed this selection very resistant to bunt and agronomically promising.

Tenmarq is the product of a cross between P1066, a strain of Crimean hard red winter wheat similar to Kanred, and Marquis. The cross was made in 1917 in the botany rust nursery for the purpose of combining the stem-rust and leaf-rust resistance of Kanred with the earliness and superior milling quality of Marquis. The variety Tenmarq originated from hybrid plant No. 16A12-5815-2, later known as Sel. No. 215421. The row originating from this plant was selected by Parker because of its high yield, apparent superior grain quality, stiff straw, and freedom from leaf rust.

Tenmarq is susceptible to bunt, while Oro is resistant, as shown by several years' results.

TABLE 1.—*Comparison of Oro and Tenmarq in regard to reaction to bunt at Manhattan, Kansas*

Variety	Annual incidence of bunt					Average bunt incidence
	1929	1930	1931	1932	1933	
Tenmarq .....	<i>Per cent</i> 46.0	<i>Per cent</i> 26.1	<i>Per cent</i> 23.3	<i>Per cent</i> 57.0	<i>Per cent</i> 33.5	<i>Per cent</i> 37.2
Oro .....	1.1	1.8	0.1	5.0	0.4	1.7

Tenmarq has been rather widely tested at other experiment stations and has been grown in cooperative experiments on Kansas farms since 1927. In 1932 the Kansas Crop Improvement Association approved Tenmarq as a standard variety of hard red winter wheat for certification, and about 520 bushels of seed were distributed to farmers in August, 1932. About 6,000 bushels of certified seed of Tenmarq were produced in Kansas in 1933.

### Hybrid Populations

Hybrid populations of Oro  $\times$  Tenmarq used in these studies consisted of 2  $F_2$  populations, each from an  $F_1$  plant, 16  $F_3$  lines, and 183  $F_4$  rows. Individual plant selections of  $F_2$  and  $F_3$  hybrid material were made for the  $F_3$  and  $F_4$  generations, respectively, on the basis of number and uniformity of tillers, stiffness of straw, weight of heads, and apparent vigor.

Each plant was numbered and threshed separately. Notes were taken on the threshed grain and further selection was made on the basis of plumpness, weight in grams per plant, corneous texture, and freedom from yellow-berry.

The studies on the inheritance of resistance to bunt in 1932 were divided into 2 parts: (a) a detailed study of the reaction of  $F_2$ ,  $F_3$ , and  $F_4$  hybrid selections when inoculated with bunt designated as physiologic form 1, from Lincoln County, Kansas; and (b) determination of the relative resistance of  $F_4$  selections when inoculated with a composite of 19 collections of bunt representative of Kansas. This composite includes 7 physiologic forms identified by Melchers,<sup>3</sup> but the larger number of collections have been grouped in the class designated as physiologic form 1.

For the studies in (a), the seed was thoroughly blackened with bunt spores of form 1, propagated on Kanred the previous season. The seed was space-sown approximately 3 inches apart in rod rows. Parental check rows were sown every 10th row. The entire nursery was sown the same day, so that soil moisture and temperature conditions were very similar for all of the selections.

The soil moisture was calculated from soil samples taken at a depth of approximately 2 inches, which was the normal depth at which the nursery was seeded. The moisture percentages varied from 15.4 per cent at one end of the series to 19.4 per cent at the other.

A continuous soil temperature record was obtained with a soil thermometer that recorded the temperature at a depth of about 2 inches. The soil temperature for the week following sowing varied from 42° to 70° F. with an average of 58.8° F. for this period. The lowest temperatures were recorded during the first few days after planting.

At harvest, the plants in each row were pulled and separated into 3 lots,

<sup>3</sup> Unpublished data.

as follows: (1) Those in which the plants were totally bunted; (2) those partially bunted; (3) those that were bunt-free.

Gaines (11), in dealing with partially smutted plants, used the formula,  $ab + c = d$ . In this formula  $a$  = the percentage of bunted heads on a partially bunted plant,  $b$  = the percentage of partially bunted plants in a row,  $c$  = the percentage of entirely bunted plants in a row, and  $d$  = the total percentage of the row that is bunted. This gives a satisfactory quantitative measure, which he believes important in studying resistance. Briggs (3), however, objects to this method because it does not indicate the nature of segregation.

In the experiments here reported all smutted plants, regardless of the degree of smutting, were recorded as totally diseased in calculating the bunt percentages used in the study of the inheritance of bunt resistance.

The experiments under (b) deal with 183  $F_4$  selections from individual  $F_3$  plants inoculated with a composite of Kansas bunt forms. These selections were made at random as far as resistance or susceptibility to bunt was concerned. This series was seeded in 5-foot rows 1 foot apart. The soil moisture in this plot at time of planting was 17.7 per cent, while the soil temperature was about the same as that for the space-planted series.

Since no selections were to be made from this section of the nursery, 4 feet of each row in this series were cut during June and the bunt counts made as soon as all the heads were in bloom so that the diseased heads could easily be identified. Plants in 1 ft. of each row were left standing until a heavy leaf-rust infection had reached full development. Leaf-rust notes were taken by C. O. Johnston and the writer.

To provide material for the experiments in 1933, individual plant selections were made in the summer of 1932, just as in the previous season. The individual plants were threshed and grain notes taken. Individual plants in the most resistant lines within each of the 3 generations showing desirable grain characters were selected for sowing. In addition several plants in rows showing approximately 20, 40, 60, and 80 per cent infection in 1932 were selected from the  $F_3$  and  $F_4$  material for sowing. These lines were inoculated with a composite of Kansas bunt forms and space-planted in 8-ft. rows.

Because of the dry weather at harvest time, the plants could not be pulled, so the bunt percentages are based on total number of culms and bunted heads rather than on the percentage of bunted plants.

#### EXPERIMENTAL RESULTS

##### Bunt Reaction of Parental Varieties and Hybrids to Physiologic Form 1

One physiologic form of the bunt organism was used to study the manner of inheritance of resistance to bunt in hybrid populations of Oro  $\times$  Ten-

marq. Parental check rows of Oro had an average of 7 per cent of bunt. Tenmarq showed an average of 43 per cent. The distribution for parents and hybrid progenies into 5 per cent classes for bunt infection is shown in table 2.

TABLE 2.—*Distribution of rows of Oro, Tenmarq, and their hybrid progenies into 5-per-cent classes for infection produced by physiologic form 1 of Tilletia levis*

Bunt	Parents		Hybrids		
	Oro	Tenmarq	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>
<i>Per cent</i>					
0 .....	1			2	66
0-4.9 .....	2			4	17
5-9.9 .....	5			1	11
10-14.9 .....	3		1	4	8
15-19.9 .....	1			2	13
20-24.9 .....		1	1	1	18
25-29.9 .....		2		1	5
30-34.9 .....		3			5
35-39.9 .....		1			4
40-44.9 .....		5			5
45-49.9 .....				1	7
50-54.9 .....		1			2
55-59.9 .....				1	6
60-64.9 .....		1			6
65-69.9 .....					6
70-74.9 .....					1
75-79.9 .....					2
Ave. per cent .....	7	43	16.8	15	18.2
Total No. rows .....	12 <sup>a</sup>	14	2	17	182 <sup>a</sup>

<sup>a</sup> Percentage bunt for 1 row was not recorded.

These hybrid populations previously had never been infected with bunt, but selection for desirable agronomic characters had been carried on in each generation.

Two F<sub>2</sub> populations from individual F<sub>1</sub> plants in 1931 had averages of 20 per cent and 13 per cent of bunt. These are intermediate between the 2 parents in bunt infection. It is of interest to note that these hybrid populations each contain only about 1 per cent of totally smutted plants. Of the parental rows grown immediately adjacent to these F<sub>2</sub> populations, the resistant parent, Oro, shows 0 per cent, while Tenmarq, the susceptible parent, shows 12.5 per cent of totally smutted plants.

The F<sub>3</sub> population consisted of 17 lines from individual F<sub>2</sub> plants grown in 1931. Two of these F<sub>3</sub> lines showed no bunt whatever, and 4 showed



infection ranging from 0 to 5 per cent. Two  $F_3$  rows showed greater susceptibility than the average of the Tenmarq parent. The greater number of bunt-infection percentages are grouped about the mean of the Oro parent. The number of  $F_3$  lines is far too small for genetic analysis.

The  $F_4$  population is made up of 183 lines from individual plants selected from 23  $F_3$  lines grown in 1931. Sixty-six rows showed 0 per cent bunt, while an additional 17 rows showed bunt infection no greater than 0 to 5 per cent. Thirty-one rows showed a greater susceptibility than the average of the Tenmarq parent. The larger number of bunt-infection percentages are grouped near the mean percentage of bunt infection of Oro, the resistant parent.

The distribution of bunt infection percentages of  $F_4$  progenies, grouped according to  $F_3$  parental lines, is shown in figures 1, 2, and 3. The dots on the base line are used to indicate 0 per cent of bunt infection. The pedigree numbers show the number of  $F_4$  progenies from each  $F_3$  line designated by the 1931 row numbers. Within each  $F_3$  family the  $F_4$  progenies are arranged in order from that with the lowest to that with the highest average percentage of bunt. The average per cent of infection of the  $F_4$  lines from each  $F_3$  family is shown by a line designated as such in the figures.

Resistant  $F_4$ -progeny rows from 8  $F_3$  lines, which were evidently homozygous for resistance to bunt, are included in figure 1. The  $F_4$  lines from the 8  $F_3$  families range from an average of 0 per cent of bunt to 1 per cent, with a maximum of 8.5 per cent for one  $F_4$  progeny row.

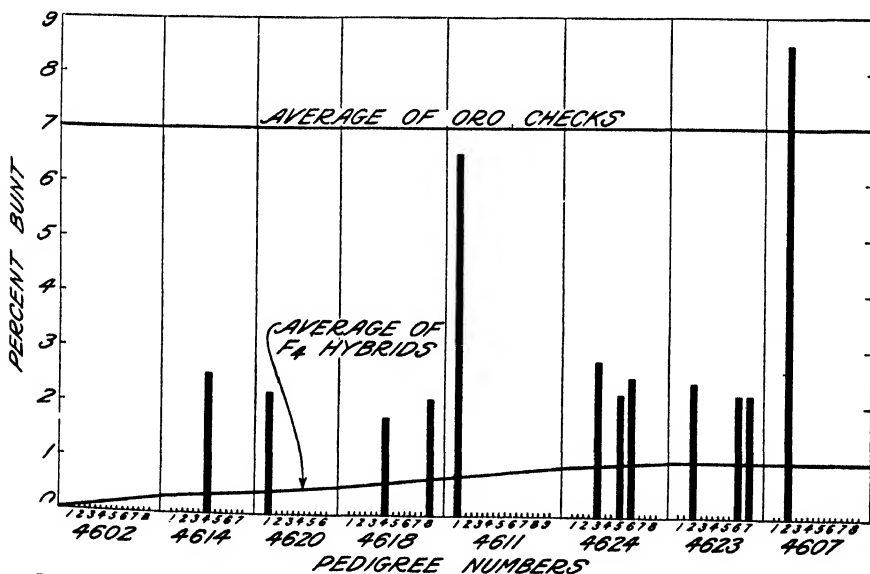


FIG. 1.  $F_4$  lines of Oro  $\times$  Tenmarq from resistant  $F_3$  lines inoculated with physiologic form 1 of *Tilletia levis*.

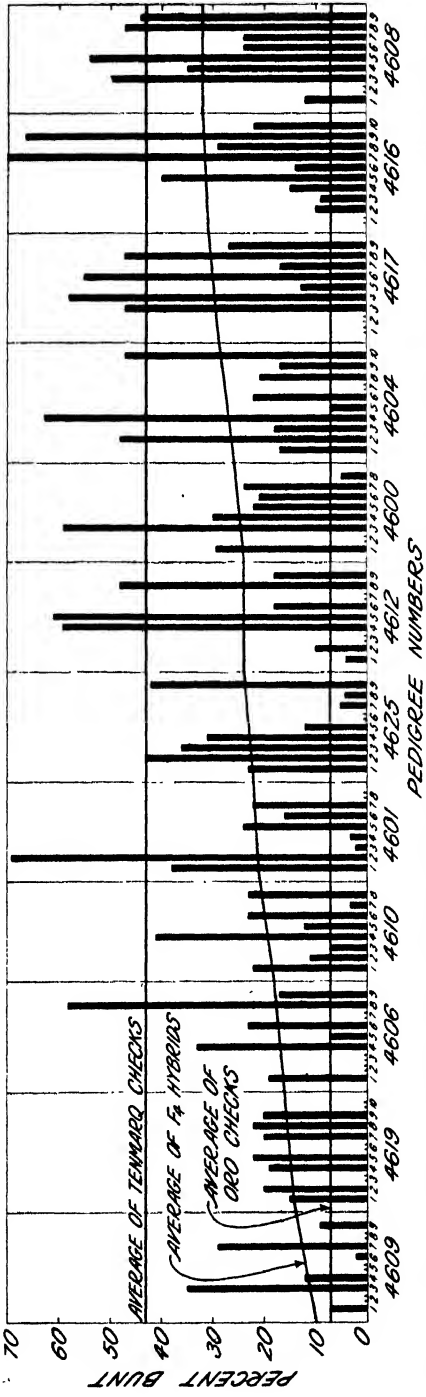


Fig. 2. F<sub>4</sub> lines of Oro x Tenmarq from F<sub>2</sub> lines heterozygous for bunt resistance, inoculated with physiologic form 1 of *Tilletia levis*.

Resistant and susceptible  $F_4$  lines from 12  $F_3$  lines that were evidently heterozygous for bunt reaction are grouped in figure 2. The  $F_4$  lines from  $F_3$  lines included in this figure range from an average of 10 per cent to 32 per cent of bunt infection. Individual  $F_4$  lines within the  $F_3$  groups range, for the most part, from 0 bunt infection to more than the average for the susceptible Tenmarq parent. There is unmistakable evidence of clear-cut segregation for bunt resistance among the  $F_4$  lines from each of the  $F_3$  lines here represented.

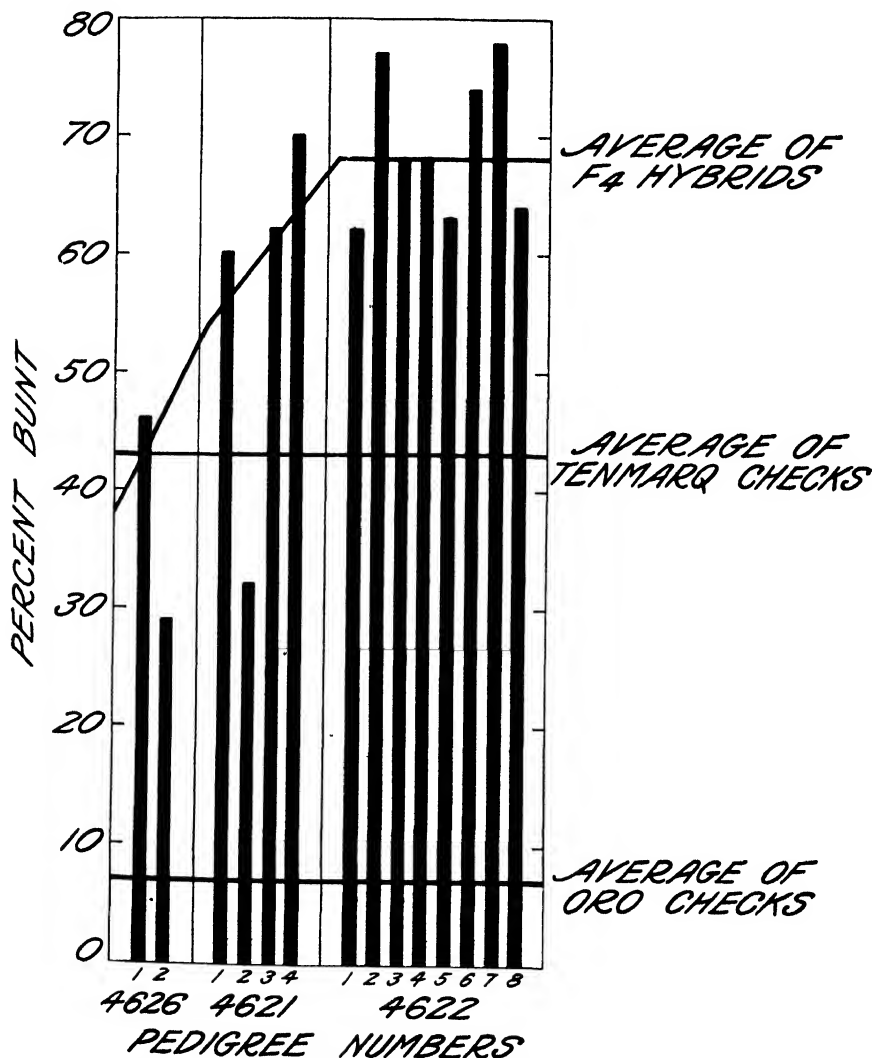


FIG. 3.  $F_4$  lines of Oro  $\times$  Tenmarq from susceptible  $F_3$  lines inoculated with physiologic form 1 of *Tilletia levis*.

Susceptible  $F_4$  lines from susceptible  $F_3$  lines are represented in figure 3. These  $F_3$  lines evidently were homozygous for susceptibility, although the number of  $F_4$  lines representing 2 of the  $F_3$  lines is rather small. The  $F_4$  lines representing  $F_3$  groups show an average bunt infection of 38, 55, and 69 per cent, respectively, for form 1. These lines show a higher bunt infection when inoculated with the composite of bunt collections.

As shown in these 3 figures representing  $F_4$  lines, the parental  $F_3$  lines may be classed as homozygous-resistant, heterozygous, and homozygous-susceptible, in the proportion of 8:12:3, respectively. The number of  $F_3$  lines represented is too small for genetic analysis, though high susceptibility evidently is recessive in this cross.

#### Reaction of Parental Varieties and Hybrids to a Composite of Kansas Bunt Collections, 1932

When inoculated with a composite of Kansas bunt forms, Oro has an average of 5 per cent and Tenmarq of 57 per cent of bunt. The distribu-

TABLE 3.—*Distribution of bunt infection percentages of Oro, Tenmarq, and  $F_4$  lines into 5-per-cent classes when inoculated with a composite of Kansas bunt forms*

Bunt	Oro	Tenmarq	$F_4$ hybrids
<i>Per cent</i>			
0 .....			37
0-4.9 .....	6		45
5-9.9 ..	4		6
10-14.9 ..			16
15-19.9 .....			14
20-24.9 ..			9
25-29.9 ..			6
30-34.9 ..			5
35-39.9 ..		2	2
40-44.9 ..		1	3
45-49.9 ..		1	2
50-54.9 ..		2	4
55-59.9 ..		1	4
60-64.9 ..			4
65-69.9 ..			5
70-74.9 ..		1	6
75-79.9 ..		1	6
80-84.9 ..			5
85-89.9 ..			2
90-95 .....		1	1
Ave. per cent bunt .....	5	57	21.9
Total No. rows .....	10	10	182*

\* No count is recorded for one row.

tion of bunt infection percentages for parents and  $F_4$  lines is shown in intervals of 5 per cent bunt infection in table 3.

Of the 183  $F_4$  progeny rows from 23  $F_3$  lines, 37 showed 0 per cent of bunt, while an additional 44 fell within the interval, 0 to 5 per cent. Thirty rows showed a greater susceptibility than the average of the Tenmarq parent.

The average per cent of bunt infection for these lines inoculated with a composite of Kansas bunt forms is very similar to that in which form 1 was used, although, as might be expected, the average infection percentages with the composite bunt are slightly higher than those obtained from form 1. The correlation surface and coefficient,  $r = 0.8875$ , for the 2 sets of data are shown in table 4. In the greater number of lines, infection percentages are low for both form 1 and the composite bunt. Deviations are usually in the direction of lower percentages of infection for form 1 and higher for the composite of Kansas bunt.

It is of interest to note that 30  $F_4$  lines show zero bunt infection to both form 1 and the composite. An additional 49 rows come within the interval 0 to 5 per cent bunt for both sources of inoculum.

#### Reaction of Parental Varieties and Hybrids to a Composite of Kansas Bunt Collections, 1933

The bunt percentages of each resistant family as observed in 1932 and 1933 are shown in table 5.

The average per cent of infection in 1933 for Oro is 0.4 and for Tenmarq 33.5, as compared with 5.0 and 57.0 per cent respectively for 1932.

A number of zero and near zero rows were obtained in the  $F_3$  generation, which showed 20 and 13 per cent bunt in the  $F_2$  generation in 1932. On the other hand, bunt infection percentages of several  $F_3$  lines were higher than those of the parent  $F_2$  cultures. This is evidence that these  $F_2$  populations segregated for bunt resistance, with the larger number of segregates showing resistance to bunt.

The  $F_4$  and  $F_5$  hybrid selections, highly resistant in 1932, continued to show very little or no infection in 1933. Since the infection percentages for the parental rows are somewhat lower in 1933, a number of the hybrid rows may later show a small amount of infection during seasons more favorable for the development of the bunt organism.

The percentages of bunt in 1933 from rows showing different degrees of susceptibility to bunt in 1932 are shown in table 6.

Results in the preceding table indicate that most of the rows showing susceptibility to bunt in 1932 are still segregating, as seems evident from the zero and other low bunt-infection percentages. In the selection of smut-free plants for sowing, the more resistant ones evidently were selected, since

TABLE 5.—(Continued)

1933 Row No.	Number of rows 1933	Per cent bunt, 1933												Per cent bunt 1932		
		0	0-5	5-10	10-15	15-25	20-25	25-30	30-35	35-40	40-45	45-50	50-55		55-60	Ave.
4221	8	8													0.0	0.4
4224	5	5													0.0	0.0
4228	3	3													0.0	1.6
4232	3	3													0.0	0.9
4233	2	2													0.0	0.8
4234	2	2													0.0	0.8
4235	3	3													0.0	0.0
4236	3	3													0.0	2.5
4237	3	3													0.0	0.8
4251	5	5													0.0	0.0
4252	3	3													0.0	0.3
4261	3	3													0.0	0.0
4262	3	2	1												0.4	0.7
4263	4	4													0.0	1.8
4264	4	4	1												0.0	1.6
4265	2	1	1												0.4	0.0
4266	3	3													0.0	0.7
4267	6	6													0.0	15.0
4269	4	4			3										12.4	0.3
4272	6	6													0.0	0.0
4275	3	3													0.0	0.0
4279	3	2	1												1.5	2.2
4281	9	9													0.0	0.8
4282	3	3													0.0	0.0
4283	1	1													0.0	0.0
4284	4	4													0.0	0.0
4285	3	3													0.0	2.0
4301	2	2													0.0	2.0
4302	3	3													0.0	2.4
4303	8	7	1												0.7	0.7
4304	4	4													0.0	1.2
4305	5	5													0.0	2.2
4306	4	4													0.0	2.2
4307	4	4													0.0	0.0
4309	1	1													0.0	0.0
4312	1	1													0.0	0.0
4315	1	1													0.0	0.0
4316	3	3													0.0	0.0
4323	4	3	1												0.2	0.0

\* These percentages are not included in the average as the total number of culms was too small. The stand was reduced by dry weather and chinch bugs.

TABLE 6.—Percentages of bunt infection of  $F_4$  and  $F_5$  Oro  $\times$  Tenmarq hybrids which showed susceptibility to bunt in the  $F_3$  and  $F_4$  generations

Row No. 1933	Per cent bunt 1932	Per cent bunt 1933
	$F_3$ lines	$F_4$ lines
3105 .....	55.0	25.7
3106 .....	49.4	2.8
	$F_4$ lines	$F_5$ lines
3113 .....	23.9	0.0
3125 .....	21.5	0.0
3133 .....	23.4	8.7
3137 .....	19.6	5.7
3173 .....	48.0	8.2
3282 .....	45.7	29.8
3271 .....	58.7	39.0
3136 .....	64.3	20.0
3269 .....	91.0	17.0
3281 .....	87.7	5.5
3351 .....	83.8	39.2
3352 .....	86.7	38.6

none of the seed from partially smutted plants was saved. These results are not entirely conclusive, however, as a number of plants from one susceptible row were not planted. It would seem that if pure-line selections were made in these generations for bunt resistance, plants selected at random from such pure lines should produce approximately the same percentages of infection. The variation in the results obtained may be due to unfavorable weather conditions, since the Tenmarq checks did not show a high percentage of infection in every case.

#### Reaction of Parental Varieties and Hybrids to a Natural Infection of Leaf Rust, 1932

A natural infection of leaf rust occurred on the  $F_4$  hybrids of Oro  $\times$  Tenmarq grown in 1932.\* Leaf-rust notes were taken on plants in a foot length of each row in the composite bunt series left standing in the field for this purpose.

The Oro parent is highly susceptible to leaf rust, while Tenmarq is only moderately so. The  $F_4$  lines of Oro  $\times$  Tenmarq show a wide range of infection with evidence of transgressive segregation. A number of the  $F_4$  lines show very high resistance to leaf rust. The data on leaf-rust infection are summarized in table 7.

A study was made of the relation of percentage of leaf rust and bunt among the  $F_4$  lines of Oro  $\times$  Tenmarq.

\* Conditions were very unfavorable for leaf rust infection in 1933.

TABLE 7.—*Distribution of leaf-rust infection of parents and F<sub>4</sub> lines of Oro × Tenmarq, into 5-percent classes*

Per cent of rust	Oro	Tenmarq <sup>a</sup>	F <sub>4</sub> hybrid
5-9.9 .....			16
10-14.9 .....			47
15-19.9 .....			6
20-24.9 .....			20
25-29.9 .....		1	6
30-34.9 .....			
35-39.9 .....			
40-44.9 .....		3	2
45-49.9 .....		3	2
50-54.9 .....			
55-59.9 .....			
60-64.9 .....			
65-69.9 .....	1		9
70-74.9 .....			
75-79.9 .....			
80-84.9 .....	6	2	13
85-89.9 .....	3		28
Ave. per cent rust .....	80	48.9	37.2
Total No. rows .....	10	9	139 <sup>b</sup>

<sup>a</sup> One row showed segregation for leaf rust.

<sup>b</sup> Rows that were segregating for resistance to leaf rust are not included.

In some F<sub>4</sub> lines the percentages of both leaf rust and bunt are high, while other lines are resistant to both diseases. Some lines are resistant to leaf rust and susceptible to bunt; others show a reciprocal reaction. Selections from F<sub>4</sub> rows resistant to leaf rust and bunt are of particular interest and value.

The correlation coefficient ( $r = 0.0151 \pm 0.5760$ ) indicates the absence of correlation between rust and bunt percentages in these F<sub>4</sub> lines.

#### DISCUSSION

These studies suggest the presence of several factors in the inheritance of resistance to bunt in the cross, Oro × Tenmarq. The number of zero and near-zero bunt lines observed in F<sub>3</sub> and F<sub>4</sub> progenies indicates that high susceptibility to bunt is recessive in this cross. Although Tenmarq is susceptible, it may carry one or more factors for resistance that differ from those for resistance carried by Oro, the resistant parent.

The number of F<sub>3</sub> lines was far too small to permit a genetic analysis of resistance to bunt in this cross. The behavior of the F<sub>4</sub> lines, therefore, has been used to determine as nearly as possible the manner of inheritance



of bunt resistance. The results suggest that in this cross a number of factors are operative in governing reaction to bunt. There is evidence of the presence of complementary factors governing reaction to bunt. The large number of zero lines suggests that Tenmarq may carry a factor or factors for resistance that, when combined with factors for resistance in Oro, produce greater resistance in some of the hybrids than that expressed by the resistant Oro parent.

The larger number of  $F_3$  and  $F_4$  lines are grouped near the average bunt percentage of the resistant Oro parent, which suggests that in this cross resistance to bunt is partially dominant and that high susceptibility is recessive.

On the basis of  $F_4$  tests, the  $F_3$  lines may be grouped into an 8:12:3 ratio, representing homozygous-resistant, heterozygous, and homozygous-susceptible families, respectively. The number of  $F_3$  lines was small, and this is an abnormal ratio and cannot be given much weight in interpreting the results.

Previous selection on the basis of plant and kernel characters has been practiced. This may have affected the bunt reaction observed in  $F_4$ , although, as far as known, the selection was random for bunt resistance. Any association between reaction to bunt and other characters would tend to cause considerable deviation. However, no association between the resistance to bunt and other characters has been observed.

The high correlation between reaction to physiologic form 1 and the composite of bunt forms suggests that possibly there is only one form of bunt of importance in Kansas, as far as the cross Oro  $\times$  Tenmarq is concerned. However, several rows show considerably greater susceptibility to the composite bunt, and any deviation usually is in the direction of higher bunt infection in the composite series. Physiologic form 1 was represented to a much greater extent than any other form in this composite inoculum, and this form probably caused a large part of the infection. It is possible that only occasionally a row or plant was infected with another form, present in smaller quantity, but sufficiently concentrated to cause infection.

Oro is very susceptible to leaf rust; Tenmarq is only moderately so. Transgressive segregation for resistance to leaf rust indicates that both parents carry factors for resistance to this disease. The plants of both parents were rather uniformly and characteristically infected. Hence it seems evident that the rows with low leaf-rust infection are really resistant and did not merely escape infection. Certain groups of  $F_4$  lines from particular  $F_3$  lines were uniformly resistant to leaf rust; others were susceptible. The inheritance of leaf-rust resistance in the Oro  $\times$  Tenmarq cross, can probably best be explained on a multiple-factor basis.

## SUMMARY

A study of the inheritance of resistance to bunt (*Tilletia levis*) was made in  $F_2$ ,  $F_3$ , and  $F_4$  generations of the cross, Oro  $\times$  Tenmarq. The resistance to leaf rust (*Puccinia triticina*) was studied in the  $F_4$  generation.

The results indicate that high susceptibility to bunt is recessive in this cross.

$F_4$  lines were grown that showed greater resistance to bunt than the resistant Oro parent, indicating that the susceptible Tenmarq parent may carry a factor or factors for resistance to bunt.

The inheritance of resistance to bunt in this cross probably is governed by multiple factors.

The correlation coefficient of  $r = 0.8875 \pm 0.0106$  between the reaction of the hybrid selections to physiologic form 1 and the reaction of the same selections to a composite of Kansas bunt collections is very high.

Transgressive segregation was noted in a study of leaf-rust reaction, indicating that factors for resistance to leaf rust probably are present in both parents.

No correlation was found between bunt and leaf-rust infection in the Oro  $\times$  Tenmarq hybrids.

A number of rows in 1932 and 1933 appeared to show a combination of the superior qualities of the Oro and Tenmarq parents and indicate that further selection should yield a bunt-resistant variety of considerable agronomic value.

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# GROWTH OF MICROORGANISMS AT AND BELOW 0° C.

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## INTRODUCTION

The growth of microorganisms at freezing and subfreezing temperatures is not only of general biological interest but also of considerable practical importance, particularly as it concerns the preservation, storage and handling of foods. With the recent rapid development of the frozen-pack food industry, involving vegetables and fruits, as well as fish and meats, the matter has taken on added significance. In an earlier paper one of the writers (6) reported on studies conducted in these laboratories in which it was shown that the careful selection of the proper storage temperature may determine, to a large extent, the fate of the microorganisms present on the frozen products. Thus, under certain conditions, very moderate cold storage temperatures ( $-2^{\circ}$  and  $-4^{\circ}$  C.) resulted in greater destruction than that occurring at a considerably lower temperature ( $-20^{\circ}$  C.). This emphasizes the need for accurate information on the minimum growth temperatures of the bacteria and other microforms normally present on raw food materials to serve as a guide to the best refrigeration practices for these products. The purpose of the present paper is to record the results of studies along this line on microorganisms derived from frozen-pack fruits and vegetables in these laboratories and to bring together from the literature the reported observations of other workers bearing directly on this subject.

Following the important early observations of Burdon-Sanderson (17) in 1871 that all bacteria are not destroyed by exposure to freezing temperatures, a very considerable amount of work has been done on the low-temperature relations of microorganisms by various research workers in different countries. Since, however, the bulk of this work has been concerned with the destruction-survival phases rather than with the actual growth of the organisms at freezing and subfreezing temperatures, it is beyond the scope of the present paper to review all of it in detail. Suffice it to say that, on the whole, the findings have shown a very marked destructive action of cold, the germicidal effect being particularly noted during the early stages of the refrigerating process, and continuing, though at a diminished rate, for many months. Complete destruction, however, has been attained but rarely, even though extremely low temperatures have been employed ( $-180^{\circ}$  to  $-190^{\circ}$  C., Belli (4);  $-190^{\circ}$  and  $-252^{\circ}$  C., Macfadyen and Rowland (38), Onkiehong (43)). Alternate freezing and

thawing (Brehme (10), Rivers (48), etc.) also have been found ineffective in bringing about complete destruction of microorganisms, and survival after low temperature exposures has been noted among nonsporeformers, as well as those that produce spores, and pathogenic as well as non-pathogenic forms.

The actual multiplication of microorganisms at freezing and subfreezing temperatures has been reported by many workers, and, since their recorded observations are pertinent to the problems now in hand, it is proposed to review briefly such of these as have come to our attention. For the interest it may have from the standpoint of the development of this line of inquiry, the observations will be considered in approximately the order of their publication.

#### LITERATURE

Forster (27), in 1887, probably was the first to call attention to the growth of bacteria at freezing temperatures when he reported that phosphorescent organisms isolated from fish preserved by cold grew well at 0° C. on various laboratory culture media. In 1892 (28) he further recorded having isolated from various natural waters, foods, wastes, rubbish, and soils a number of organisms that were able to grow at this temperature. He noted also that such organisms were present in great numbers on the surface and in the intestines of freshwater fish and were especially numerous in sea water and on sea fish.

In 1888 Fischer (25) also reported the isolation of 14 different kinds of microorganisms, from harbor water and from soils, which grew at 0° C. These included phosphorescent and also nonluminous species, an unidentified fungus, a coccus, and 7 rod forms, 3 of which were fluorescent species having proteolytic properties. Fischer (26) also was probably the first to note, in 1893, the growth of certain pathogenic bacteria (cholera vibrios) at 0° C.

Conradi and Vogt (21) recorded that a "proteus bacillus," another pathogenic organism, grew slowly at this temperature.

Baur (3) and also Brandt (9), in 1902, noted the slow but positive denitrification of nitrite bouillon by *Bacterium lobatum* at 0° C.

The same year Schmidt-Nielsen (50) reported the growth at 0° C. of *Bacillus aquatilis fluorescens non-liquefaciens*, *B. granulosus*, *B. paracoli gasoformans anindolicum*, *B. radiatum*, *B. tarde fluorescens*, *B. pestis*, and *B. proteus fluorescens*, as well as a strain of *Saccharomyces pastorianus* I, a red *Torula*, and *Actinomyces ochraceus*, *A. carneus*, and *A. ochroleucus*.

Müller (42), working with sausage meat, fish, intestinal contents of fish, milk, vegetables, meal, garden soil, and muck, isolated 36 different cultures from these products, all of which grew at 0° C. From these he identified

4 strains of *B. fluorescens liquefaciens*, one of *B. fluorescens non-liquefaciens*, one of *Micrococcus flavus tardigradus* and one of *M. carneus*. Four other unnamed species of bacteria were described. In addition were included in the group a *Mucor* (*M. mucedo* (?)), a *Penicillium* (*P. glaucum* (?)), an *Oidium*, and a *Blastomyces*. He found that not only were the microorganisms capable of developing at 0° C. widely distributed but their vital manifestations were the same at 0° C. as at the higher temperatures, the difference being merely one of intensity.

Feitel (24) reported studies on denitrifying bacteria isolated from deep sea water in which it was shown that about one-third of the cultures developed slowly at 0.3° to 0° C. *Bacillus actinopelte*, *B. ornatum*, and *Bacterium balticum* were included in this group.

Klein (35) found a *Blastomyces* causing brown spots on cold-storage beef to be capable of making slight but distinct growth at 28°–30° F. (–2.22° to –1.11° C.) in 10 days. It showed no growth at 16°–18° F. (–8.89° to –7.78° C.) in this period of time, but the organism was not killed by this treatment, as normal growth appeared in 48 hours when the cultures were brought to ordinary room temperatures.

Tsiklinsky (52), in the journal of one of the French expeditions into the Antarctic regions, noted the presence of numerous microorganisms in the intestines of fishes, as well as in the sea water, although the temperature of the water was –1.5° C.

Richardson (47) stated that certain nonproteolytic bacteria were capable of developing on frozen meat. He did not get growth on frozen meats at temperatures of –9° to –12° C., however, and gave it as his opinion that growth at this temperature would be almost impossible. Emphasis was laid on the point that it is the solid state of the medium and not any specific temperature that is the limiting condition for growth and reproduction, although retardation of growth ensues with lowering of the temperature.

Eustace (22), in work on the storage diseases of apples and peaches, recorded the growth of *Penicillium glaucum* on inoculated apples held at 32° F. (0° C.) for 2 months or more, and decay in 2 weeks, in peaches held at this temperature, because of the activity of brown rot (*Sclerotinia fructigena*). *Alternaria* sp., *Glomerella rufomaculans* (bitter rot), *Sphaeropsis malorum* (black rot), *Cephalothecium roseum* (pink rot) and *Venturia inaequalis* did not cause decay at this temperature.

Pennington (44), in a study of so-called “clean” and “market” milks held at –1.67° to –0.55° C., found very marked increase in numbers of bacteria, even though the milk was semi-solid with ice. In her summary she states: “Bacterial growth at the end of a week, even in the cleanest milk, which contained as low as 300 organisms to the c.c., was pronounced. There was a steady increase in the number of organisms for 5 or 6 weeks, and at

their maximum they numbered hundreds of millions. Occasionally they passed the billion mark per c.c." Certain species of bacteria, such as *Bacillus formosus*, *B. solitarius*, and *B. Raveneli*, were especially resistant to cold and, in pure culture, frequently were the predominating species, or almost so, at the close of the experiment.

Ravenel, Hastings, and Hammer (45) made studies on the effect of storage at 0° and -9° C. on the bacterial flora of 2 grades of milk, one considered the best obtainable and the other a mixed dairy milk of fair quality. No increase in numbers of bacteria was obtained in the milks held at -9° C. for periods of 160 to 203 days. There was clumping of the casein and fat, an increase in soluble nitrogen, and a decrease in acidity. In the milks held at 0° C., however, there was a marked increase in the bacterial content, which resulted in an increase in acidity, an increase in the percentage of soluble nitrogen, and a decrease in the total nitrogen content, due, probably, to the liberation of free nitrogen.

From a series of studies on bacteria in frozen soils Conn (18, 19, 20) reported a rapid multiplication of bacteria in solidly frozen soils, the increase being due to the development of slow-growing forms distinct from those normally making up the majority of soil organisms during the summer months.

Brown and Smith (16) obtained results supporting those of Conn, just mentioned. They noted further that frozen soils possess much greater ammonifying power than nonfrozen soils, that there was an increase in nitrogen-fixing power in frozen soils, and that the denitrifying power decreased gradually with continuance of the frozen condition.

Massee (40), in studies on *Cladosporium herbarum* as the causal agent in "Black Spot" of beef, shipped in cold storage from Argentina, found that this organism grew slowly at 0° C. Growth below this temperature was appreciable only after one month.

Reed and Reynolds (46), investigating the effect of low temperatures on the growth and activities of bacteria in milk, found that at the temperature of -1° C. *Bacillus putidum*, *B. aerogenes*, *B. cyanogenes*, *B. proteus vulgaris*, *B. coli*, *B. subtilis*, *B. fluorescens liquefaciens*, *B. prodigiosus*, *Bacterium lactis acidii*, *Sarcina lutea*, *Oidium lactis*, *Microspira tyrogena*, and *M. citricus* were all able to make growth. Considerable variation was noted in the behavior of different organisms at this temperature; certain of them, for example, *Bact. lactis acidii* and others of the acid-producing group, increased at first but decreased under continued storage at this temperature, while still others, such as *Microspira tyrogena*, grew slowly at first but made greater growth as the storage period was continued. Milk, therefore, that was still low in acid and would have been considered sweet had actually undergone marked chemical change, which affected its quality.

Brooks and Cooley (11), working on the temperature relations of apple-rot fungi, found that *Alternaria* sp., *Botrytis cinerea*, *Cephalothecium roseum*, *Neofabraea malicorticis*, *Penicillium expansum*, *Sclerotinia cinerea*, *Sphaeropsis malorum*, and *Volutella fructi* grew in inoculated apples at the temperature of 0° C. *Fusarium radiculicola* and *Glomerella cingulata* only, of all the species used, failed to grow at this temperature. In commercial cold-storage experiments on rather immature fruit *Penicillium expansum* continued to develop at 0° C. in the case of delayed storage but was unable to grow at that temperature in the case of immediate storage. The spores of the species of *Alternaria*, *Botrytis*, *Penicillium*, and *Sclerotinia* had germinated in corn-meal agar at 0° C. at the end of a month but those of species of *Aspergillus*, *Cephalothecium*, *Fusarium*, *Glomerella*, and *Pestalozzia* had not. The temperature response was found to be greatly modified by the nature of the medium on which they were grown.

Vanderleek (53), in studies on the bacteria of frozen soils in Quebec, found that bacteria increased rapidly in both frozen and nonfrozen soils when abundant organic material was present for decomposition, but severe frost prevented this development. Bacteria decreased in number when the soil thawed, and increases or decreases in numbers were observed to follow generally the rise or fall in soil moisture. Attention was called to these facts also by Conn (18, 19) and by Brown and Smith (16) mentioned above. In a second paper (54) Vanderleek made the important observation that changes produced by bacteria in frozen soils are dependent on the kinds of enzymes produced by the organisms; that is, evident influence of intracellular enzymes depends upon active growth of the organism. Extracellular enzymes, however, may act in the absence of active growth.

Monvoisin (41), working with the molds occurring on cold-storage meats, reported the finding that molds, normally showing abundant white colonies from spores in 2 days and mature spores in 5 to 6 days at ordinary temperatures, required 2 to 3 months for the appearance of the white colonies and 4 to 5 months for the maturing of the spores at an incubation temperature of -9° C. on the medium he used. Spores failing to show germination at -6° and -9° C. did so on removal to temperatures of 12° to 15° C. in 24 to 36 hours and pursued normal growth. When these same cultures were returned to the low-temperature rooms the growth continued, though slowly. He emphasized the importance of the amount of water vapor in the air as influencing mold growth at various low temperatures. The molds encountered on cold-store meats, in the order of their importance, were: *Thamnidium elegans*, *Mucor mucedo*, *Rhizopus* sp., and *Penicillium glaucum*.

Brown and Halversen (15) found that the number of bacteria in frozen soil increased as the temperature became lower and fell with higher tem-



peratures, regardless of the moisture content. They noted also a decrease with thawing.

Vass (55) reported no increase in bacteria in frozen soils and gave the breaking up of clumps caused by freezing as the explanation of the findings of other workers.

Bidault (7), in a study of the molds of frozen meats, found that colonies of *Choetostylum fresenii* and *Hormodendron cladosporioides*, placed in full vegetative condition in a chamber maintained at a temperature of  $-10^{\circ}$  C., for 2 months, showed slight growth. This was more marked at the end of 2 more months, the temperature being raised to  $-8^{\circ}$  C. Other molds—*Penicillium crustaceum*, *Cladosporium herbarum*, *Botrytis rosea*—grew at temperatures between  $-6^{\circ}$  and  $0^{\circ}$  C. He ranged the various microorganisms investigated in the order of their increasing sensitiveness to cold as follows: *Choetostylum fresenii*, *Hormodendron cladosporioides*, *Cladosporium herbarum*, different species of *Botrytis*, *Thamnidium elegans*, *Penicillium crustaceum*, *Torula*, and yeast forms. These last continued their development only in the neighborhood of  $0^{\circ}$  C.

Brooks and Kidd (14) recorded the growth of *Cladosporium herbarum*, the organism associated with "Black Spot" on meats, at temperatures not rising above  $-5.5^{\circ}$  C. and stated that this was by no means the minimum for this organism. They also mentioned evidence that white molds (species of *Sporotrichum* and *Oospora*) could develop at temperatures below the freezing point during prolonged storage.

Stiles (51), in discussing the preservation of foods by freezing, noted that, while many microorganisms cannot withstand temperatures below  $-1^{\circ}$  or  $-2^{\circ}$  C., others can live and develop at temperatures considerably lower and the development of all molds is not rendered impossible by storage at temperatures below the freezing point.

Brooks and Hansford (13) found that, of the fungi developing on meat, *Cladosporium herbarum* not only grew but developed fresh spores at  $18^{\circ}$  F. ( $-7.78^{\circ}$  C.); *Sporotrichum carnis*, the organism causing white spots on meats, developed slightly at this temperature and appreciably at  $28^{\circ}$  F. ( $-2.22^{\circ}$  C.); *Thamnidium* sp. developed at  $28^{\circ}$  F. and slightly at  $18^{\circ}$  F. ( $-7.78^{\circ}$  C.); *Mucor* sp. developed fairly well at  $28^{\circ}$  F.; and representatives of the genus *Penicillium* developed appreciably at  $28^{\circ}$  F. They also found that the spores of *Cladosporium herbarum* germinated at  $-6^{\circ}$  C. but that subsequent growth was more rapid if germination had taken place during a short period of exposure to ordinary room temperatures. Failure to induce spores of some of the other molds to germinate at  $-6^{\circ}$  C. was thought due, perhaps, to unfavorable humidity conditions.

Wright (56) observed that when *Mucor mucedo* had been grown on meat at temperatures ranging from  $-2^{\circ}$  to  $-1^{\circ}$  C. until the growth was

well established it formed black spots on the meat when placed in cold storage at  $-12^{\circ}$  to  $-15^{\circ}$  C. Similar results were secured with *Penicillium glaucum*, except that the initial temperature of development was about  $4^{\circ}$  C. He concluded that *Cladosporium herbarum* was only one of a number of molds that might cause black spot on meats and stated that to prevent mold development in cold storage meat should be held at temperatures not higher than  $-9^{\circ}$  C.

Brooks (12), in an attempt to check the work of Wright, just mentioned, grew *Mucor mucedo* on mutton at  $-1^{\circ}$  C. until growth was well established and then stored the meat at  $-11^{\circ}$  C. for  $4\frac{1}{2}$  months. No black spots were obtained. Similar results were secured with a species of *Penicillium* developed at  $4^{\circ}$  C. and then stored at  $-11^{\circ}$  C. for a like period.

Fay and Olson (23), in a study of the bacteriology of ice-cream manufacture and storage, found that, during the first few days after placing the ice cream in the hardening room or packing it in ice or brine, there was usually a marked decrease in the number of bacteria, a decrease varying from none to 75 per cent in the first 2 days and continuing after the first week at a much slower rate. Frequently, however, after about a month, there was a slow increase in the numbers of bacteria because of growth at the prevailing low temperatures.

Bardach (1) isolated 11 different kinds of nonspore-forming putrefactive bacteria from sewage, sludge, and salt water, which were able to multiply at temperatures of  $0^{\circ}$  to  $-2^{\circ}$  C. The ordinary life processes were carried on in a normal manner at these low temperatures, though the rate of growth was slower than at higher temperatures.

Rubentschik (49) found that *Urobacillus psychrocarcticus* and *Urosarcina psychrocarcticus* were able to grow at temperatures of  $-1.25^{\circ}$  to  $-2.5^{\circ}$  C. In bouillon containing 3 per cent of urea the latter organism increased from an average of 2,870 to 28,520,000 per 1 c.c. in 25 days.

Haines (29, 30, 31, 32, 33, 34), working on some of the organisms developing under cold-storage conditions, found that *Sporotrichum carnis* grew at temperatures of  $-5^{\circ}$  and  $-7^{\circ}$  C. The lower limit of growth of this fungus on supercooled Czapek's agar was near  $-10^{\circ}$  C. Growth was never obtained on the frozen medium. He noted further that, under favorable conditions, special types of organisms could multiply at  $0^{\circ}$  C. to such an extent as to cause the spoilage of carcasses in storage. His findings indicated that "storage at  $-5^{\circ}$  C. leads to a steady fall in the numbers of bacteria, followed by a period when the numbers are at a minimum and that this in turn is followed by an increase in numbers, due mainly to the growth of yeasts and moulds." In no case did he observe visible growth of bacteria, yeasts, or molds on carcasses of mutton stored at  $-10^{\circ}$  C. or lower. Organisms of the *Pseudomonas (fluorescens)* group were found to

grow relatively well at 0° C. Experiments with various strains of *Actinomyces* showed a minimum growth temperature of 0° C. for some, while one strain grew at temperatures down to about -5° C. Various yeasts and *Torulae*, commonly present on cold-storage meat, grew comparatively well at -5° C.

Bonney (8), in the discussion of findings from experiments on frozen-pack fruits, conducted cooperatively with workers of the Bureau of Plant Industry, noted that a temperature of 30° F. permitted mold growth and that, under certain conditions, growth might take place also at considerably lower temperatures. The strength of the syrup used in the pack and the degree of vacuum within the container were found to be important growth-inhibiting factors under these conditions.

Lea (36, 37) reported that a storage temperature of -5° C. failed to prevent the growth of molds and yeasts on frozen mutton and lamb, but no visible growth occurred at -10° or -20° C. Freshly slaughtered beef stored at 0° C. and examined at intervals over a period of 42 days showed at the end of the storage period extensive mold growth, while with like samples handled in the same way, but with the air agitated by means of fans, the growth was much retarded and most of the surface was essentially free from visible growth. A carcass of lamb and a side of pork, stored under these same conditions with aeration, showed no appreciable growth of molds or yeasts after 60 days. Molds, yeasts, and bacteria developed on beef held at -1.6° C. for 42 days without air circulation.

Magoon (39) reported marked growth of many molds on apparently solidly congealed frozen-pack fruits and the production of spores by these organisms at temperatures well below 0° C. (commercial storage at about -5° C.); and Berry (5) has recorded the development of pink yeasts and molds in berries packed in paper containers and stored at 28° F. (-2.22° C.).

Because of the widespread interest in the significance of *Clostridium botulinum*, as concerned with the preservation of food products, it may not be amiss to state here that in the review of the work of these numerous investigators no published record has been found of the development of this organism at temperatures below 31° F., as reported earlier by the senior writer (5).

#### EXPERIMENTAL DATA

In the course of the present work on fruits and vegetables preserved at low temperatures specific information has been secured regarding the growth of a number of microorganisms at temperatures of -4° and -6.7° C. Details as to the types of organisms concerned, the products attacked, and the time periods required for marked spoilage to occur are here briefly presented.

Microorganisms Growing at  $-4^{\circ}\text{C}$ .

1. *Pseudomonas fluorescens*. Scalded or "blanched" peas, packed with 3 per cent NaCl solution in nonairtight containers and held at  $-4^{\circ}\text{C}$ ., showed microbial spoilage after 4 weeks of storage. Ice formation in the product had been slow. The predominating type of organism obtained by plating on nutrient agar in high dilution and incubating at  $20^{\circ}\text{C}$ . was inoculated into sterile pea broth and nutrient gelatin, which were at once put at  $-4^{\circ}\text{C}$ . for incubation. In 4 weeks unmistakable growth had taken place. On examination the organisms proved to be Gram-negative rods of medium size, nonspore-forming, motile by means of a single polar flagellum, aerobic, liquefying gelatine rapidly, reducing nitrates, not producing indol nor coagulating milk, and forming a green pigment that diffused into the medium.

The growth curve at  $-4^{\circ}\text{C}$ . of this strain of *Pseudomonas fluorescens* in 3 per cent NaCl bouillon, isolated from frozen-pack peas, is shown in figure 1.

2. *Lactobacillus* sp. Peas, prepared and stored as above, except that hermetically sealed containers were used, showed after 8 weeks an increase

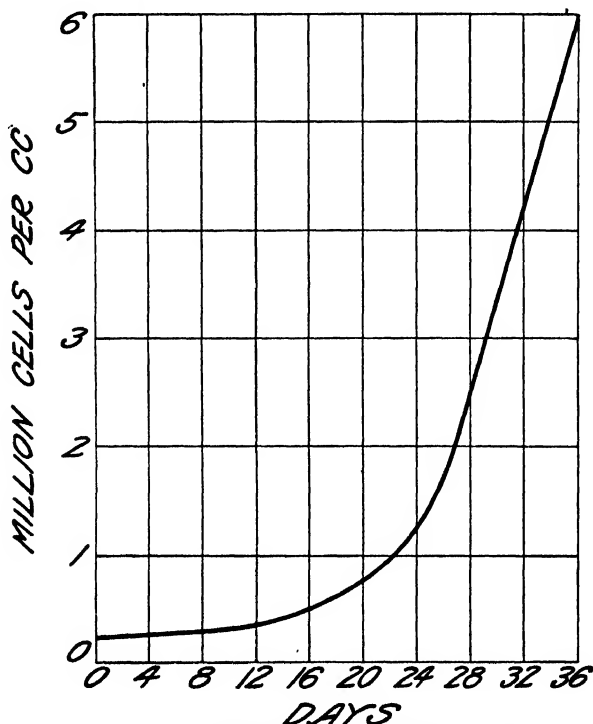


FIG. 1. Growth curve of *Pseudomonas fluorescens* at  $-4^{\circ}\text{C}$ . in 3 per cent NaCl bouillon. It was isolated from frozen-pack peas.

in bacterial content, the plates showing unusual numbers of small glistening colonies. The pH of the peas had changed from 6.5 to 6.0. As transfers from these colonies grew in gelatin a series of stab inoculations was made in this medium and the tubes incubated at  $-4^{\circ}$  C. Slight growth was evident after 6 weeks. Study of the organisms showed them to be small blunt rods, Gram-positive, nonspore-forming, microaerophilic, growing slowly on agar and gelatin, which they did not liquefy, not forming indol nor reducing nitrates, forming acid but not gas from sucrose, maltose, dextrose, lactose, and less acid with no gas from mannitol, raffinose, and trehalose.

3. *Torula* sp. Spoilage of strawberries, packed in 50 per cent sucrose solution in nonairtight containers and held at  $-4^{\circ}$  C., was noted after 4 weeks' storage, cultures in nutrient agar at this time yielding pink colonies, previously little in evidence. Inoculations from these were made to 2 per cent nutrient agar by the stab method, and growth was evident at the surface of the medium after 2 weeks' incubation at  $-4^{\circ}$  C. The organisms were mostly oval, about  $3 \times 5 \mu$ , reproduced by budding, rapidly formed pink moist growth on nutrient agar at  $18-32^{\circ}$  C., and did not ferment sucrose, maltose, dextrose, or lactose.

4. *Monilia* sp. In addition to *Torulae*, whitish patches appeared on the surface of strawberries held for 6 weeks at  $-4^{\circ}$  C. Direct examination showed oval and elongated cells with some filaments. Pure cultures having been secured by plating on wort agar, inoculations were made to tubes of wort,  $15^{\circ}$  Brix, which were incubated at  $-4^{\circ}$  C. A pellicle formed in 8 weeks. A description of the organism follows: Cells oval, measuring about  $3 \times 4.5 \mu$ , with some filamentous forms on nutrient agar, agar colonies small, circular, homogeneous, white, convex; numerous calcium oxalate crystals in the medium; wort-agar colonies about 4 mm. in diameter, mycelioid, convex, not becoming colored; hyphae septate; conidia in clusters at the sides and tips of branched conidiophores. Young wort cultures impart a fruity odor. Acid without gas is formed from sucrose, maltose, dextrose, and levulose but not from lactose.

5. *Penicillium* spp. Mold growth was observed on the outside of several paper containers of peas packed in 3 per cent NaCl solution after 3 months' storage at  $-4^{\circ}$  C. In 7 months the colonies were about 6 mm. in diameter and of a slightly greenish hue. When the containers were moved to the laboratory, the colonies—obviously species of *Penicillium*—matured in 5 days, appearing flat with powdery surfaces. Most of them were bluish-green—Type I; a few were lighter green—Type II. Some characteristics of these organisms follow:

*Type I.* Wort-agar colonies, spreading, 2 to 2.5 cm. in diameter, and 1 to 2 mm. in elevation, bluish-green, surface granular, margin zonate, re-

verse salmon orange, odor pronounced; conidiophores in part as short branches of trailing aerial hyphae, but mainly in fascicles; conidial chains 100 to 200  $\mu$  long; conidia elliptical, smooth,  $3 \times 3.5 \mu$  in dimensions. Gelatin not liquefied in 21 days. An inoculation of Type I, mostly spores, on deep wort agar, showed slight growth at  $-4^{\circ}$  C. in 50 days.

*Type II.* Same as Type I, except that the color is greener, with reverse of the colony yellow green, and gelatin liquefied in 10 days.

Both types readily produced rot in apples. Coremia of the massive type were not noted. The two strains presumably below in the *Fasciculata* section of the genus *Penicillium*, and may tentatively be placed in the *P. expansum* series.

#### Microorganisms Growing at $-6.7^{\circ}$ C.

1. *Cladosporium* sp. Okra, packed in 3 per cent NaCl solution, and kale, without packing liquid and stored in nonairtight containers at  $-6.7^{\circ}$  C. for 15 months and 16 months, respectively, showed, on the surface, numerous dirty white to olivaceous mold colonies, 3 to 4 mm. in diameter. In addition to direct examination, a series of inoculations was made at once on nutrient agar and wort agar. The colonies resulting after a week's incubation at  $20^{\circ}$  C. appeared to be of a single species. A brief description follows: Colonies spreading, flat, olivaceous, reverse black; hyphae septate, intricately branched, sooty, nodose on nutrient agar; conidiophores branched; conidia terminal and lateral, ovoid, greenish,  $3.8$  to  $6 \times 6$  to  $14 \mu$ ; the larger often bicellular.

2. *Sporotrichum* sp. Scalded peas, packed in 3 per cent NaCl solution and stored at  $-6.7^{\circ}$  C. in nonairtight containers, showed, in 10 months, numerous mold colonies on peas that projected above the frozen-pack liquor. The colonies were white, about 3 mm. in diameter, and raised to convex. Mycelial tufts were at once inoculated to nutrient agar, wort agar, and sterile peas. Equally good growth occurred in all 3 types of media in 7 days at  $20^{\circ}$  C. Following is a brief description:

Colonies, up to 1 cm. in diameter, white at first, later somewhat fawn, about 2 mm. in height; reverse yellow; hyphae septate, conidiophores short, branched, not in whorls; conidia smooth, pyriform,  $3.0 \times 4.5 \mu$ , not in chains, and borne laterally and terminally on the conidiophores.

Sterile peas in 3 per cent sodium chloride solution inoculated with small mycelial tufts and spores of the *Cladosporium* sp. and *Sporotrichum* sp. mentioned above and placed at once in storage at  $-6.7^{\circ}$  C. showed growth in 3 months. After 4 months' incubation growth was no longer confined to the peas projecting above the brine but had definitely started on the surface of the frozen brine itself.

### Media for Cultures at Subfreezing Temperatures

Since ice may be more inimical to the growth of microorganisms than the cold producing it, media intended for cultural studies below 0° C. should remain ice-free at the temperature selected. While, as might be expected, standard bouillon and agar resist physical change at the freezing point of water, experience shows that they are unreliable for long incubation at temperatures near -2° C. For studies at -4° C. it is necessary to depress the freezing point of the bouillon or agar by the addition of an adequate amount of nontoxic solute, such as 3 per cent NaCl. The age and condition of storage of the medium are modifying factors; thus, tubed nutrient gelatin, concentrated by 5 weeks' storage at 18° C. in a relatively dry room, has been used successfully at -4° C. As a corollary, any increase in the percentage of the ingredients used in a medium tends to lower the freezing point. In this connection wort,<sup>1</sup> 12° Brix, has not been found reliable at -4° C.; but, as this medium is used for mold and yeast culture, a wort of 15° Brix, which, as a rule, does not freeze at -4° C., may be employed without inhibitive plasmolytic action on the cells.

### DISCUSSION

This brief survey of investigations already made shows that freezing temperatures can by no means be relied upon to prevent the microbial spoilage of food products when those temperatures are at or even well below zero Centigrade. The organisms able to carry on at these temperatures are widely distributed and of various kinds. In the studies here reviewed no less than 15 different genera of fungi and more than 30 different species of bacteria, as well as yeasts and their close relatives, the torulae, have been found able to grow and function normally under these low-temperature conditions. To be sure, in most cases this growth has been relatively slow, but when considered in relation to practical refrigeration practices in which storage for long periods is common, it becomes of very real significance. It is of particular value to know that the complete life cycle of some food-destroying fungi, from spore germination through the vegetative phases of growth to complete spore production, may take place at temperatures as low as -7.78° C., that spore production may take place at -9° C., and that vegetative growth and food deterioration induced thereby may proceed at a temperature of -10° C. or, possibly, even somewhat below this point.

The physical condition of the refrigerated material and the environmental factors seem to be more important than the degree of cold in de-

<sup>1</sup> This useful medium is readily prepared from commercial malt syrup. The unadjusted reaction is approximately pH 4.6.

termining whether or not growth will take place. The mere presence of ice in the product is no criterion of thorough preservation. In the case of molds, at least, the presence or absence of free oxygen and, to a considerable degree, the amount of moisture available are important determining factors. The favorable effect of sugar or sugar syrup in frozen-pack fruits in preventing mold growth probably is due to its high osmotic or dehydrating action.

It is apparent that under the conditions of moderate cold, *e.g.*,  $-7^{\circ}$  to  $-2^{\circ}$  C., the majority of microorganisms are killed but that a few survivors will remain that, in the absence of inhibiting conditions, are able to develop. These cold-tolerant organisms may or may not be prominent in the original microflora. Incidentally, this adaptability of microorganisms to low-temperature conditions has its parallel in the ability of certain individuals, not true thermophiles, in many microbial populations to adapt themselves to relatively high temperature conditions. It would appear from the data reviewed that the lower temperature limit for growth lies close to  $-10^{\circ}$  C., though the report of Bonney (8) suggests that under certain conditions the minimum temperature may be even lower than this.

It is important to note that among these organisms recorded as growing at zero Centigrade are included some of the pathogenic forms.

The direct findings of Brooks and Cooley (11) to the effect that mold growth that is prevented by prompt refrigeration may proceed at the same storage temperature if refrigeration be delayed, together with the findings of other workers (41, 13, etc.) that vegetative growth of some molds may proceed at temperatures that prevent the germination of their spores, and the observation (54) that enzymes continue to function after the growth of the cells that produced them has been inhibited by cold, all emphasize the practical importance of prompt handling and refrigeration of those products intended for frozen-pack storage. Such prompt handling and storage add materially to the chances for successful preservation.

The increase in the numbers of organisms in frozen soils, attributed by several workers to the development of organisms especially adaptable to low-temperature conditions, might be due, it would seem, to changes in the food material—the formation of ice resulting, perhaps, in a more favorable concentration of the nutrients or in alteration in its composition. The findings of Barnes (2) that the growth and photosynthesis of *Spirogyra* are greatly accelerated and the multiplication of Protozoa is more rapid in cultures rich in trihydrol, that molecular form of water particularly abundant in freshly melted ice, are very suggestive as bearing on this point. The possibility of lessened antibiosis should also be considered in this connection.

It is of interest to note that of the various microorganisms growing at



or below 0° C. none thus far investigated can properly be, classed as "psychrophiles." Müller (42), among others, has called special attention to the fact that this designation is a misnomer, and the findings in the present work confirm his conclusions; for while many organisms are able to grow at these low temperatures, it is apparent that the growth conditions are not optimal, as all develop more rapidly at temperatures well above the freezing point. They appear, therefore, to be a group of mesophilic organisms that are able to carry on relatively slowly at a lower temperature range than the majority of microforms.

#### SUMMARY

A review of the literature on the minimum temperature requirements of microorganisms shows that:

Since 1887, numerous species, including even some of the pathogenic forms, have been observed to grow at 0° C. and, in some cases, considerably below that temperature. In general, the organisms developing at subzero temperatures are fungal rather than bacterial, though some bacterial growth has been observed.

Of those organisms developing at temperatures below -5° C. species of *Cladosporium* and *Sporotrichum* have been most commonly observed.

In general, following exposure to moderately low temperatures, *e.g.*, -5° to -6° C., there is a marked reduction in the number of viable organisms. Under aerobic conditions, however, this initial decrease may be followed by an actual increase of cold-tolerant molds.

There is evidence that the development of molds may take place at temperatures below those at which their spores can germinate. Spore germination of certain molds, however, has been observed at temperatures as low as -7.78° C.

Any microbial growth below -10° C. seems unlikely.

Failure of an organism to grow at a subzero temperature may be due to ice formation in the medium rather than to the effect of cold alone, though apparently some microforms, particularly certain molds, are less affected by ice formation than others.

Humidity and oxygen relationships are factors to be evaluated in a consideration of microbial growth at low temperatures.

No reported record has been found of the growth of *Clostridium botulinum* at subfreezing temperatures.

The writers' records show growth at -4° C. of *Pseudomonas fluorescens* and species of *Lactobacillus*, *Torula*, *Monilia*, and *Penicillium*. Growth of species of *Cladosporium* and *Sporotrichum* at -6.7° C., also, is reported. These organisms were isolated from frozen-pack fruits and vegetables and grown in pure cultures at the temperatures mentioned.

A graph shows the rate of growth of a strain of *Pseudomonas fluorescens* at  $-4^{\circ}$  C.

Types of culture media suitable for use in the study of microbial growth at temperatures below  $0^{\circ}$  C. are considered.

The contention that a truly cold-loving or "psychrophilic" group of microorganisms exists appears unwarranted by available evidence. While organisms recorded as growing at  $0^{\circ}$  C. or below may with propriety be regarded as forming a cold-tolerant group, their temperature requirements for best growth definitely place them in the mesophilic class.

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# THE COMPARATIVE BEHAVIOR OF FOUR CLOVER-LEAF PARASITES ON EXCISED LEAVES<sup>1</sup>

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## INTRODUCTION

In the course of inoculations with a number of clover pathogens on excised leaves it was observed that incubation conditions favorable to some fungi were unsuitable for the development of others. One important factor, as shown in this paper, was found to be the nutritional condition of the excised leaves.

The literature, prior to 1924, on the use of excised leaves for infection experiments with parasitic fungi, especially the rusts, has been reviewed by Clinton and McCormick (1). Mains (3) was perhaps the first to show the value of a sugar nutrient solution in such studies. Waters (7) controlled the formation of urediniospores and teliospores of a number of rust fungi by manipulating the carbohydrate supply of excised leaves. Trelease and Trelease (6) used excised leaves in demonstrating the importance of available sugars for the development of wheat mildew. Of a number of sugars tested Waters found sucrose the most suitable, but Trelease and Trelease found sucrose, dextrose, and glycerine about equally valuable.

Salmon (5) was perhaps the first to use excised clover leaves for infection experiments. He inoculated such leaves with powdery mildew in moist Petri-dish chambers and reported successful infection. Clinton and McCormick inoculated excised clover leaves, floating on water, with rust and they state that, "With such tender leaves as clovers, however, death of the leaves often occurred too early to secure definite results." Waters secured satisfactory infection with rust on excised leaves of alsike clover.

## MATERIALS AND METHODS

Four foliage diseases of red clover, *Trifolium pratense* L., were studied on excised leaves, namely: rust caused by *Uromyces fallens* (Desm.) Kern., mildew caused by *Erysiphe polygoni* DC., leaf spot caused by *Macrosporium sarcinaeforme* Cav., and anthracnose caused by *Colletotrichum trifolii* S. M. Bain. The first two of these diseases are caused by obligate parasites

<sup>1</sup> Joint contribution from the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Botany Department, Purdue University Agricultural Experiment Station.

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and the latter two by facultative parasites. Pathogenicity studies with *Cicinnobolus cesatii* (syn. *Ampelomyces quisqualis*), the common parasite of powdery mildews, have also been conducted on excised mildew-infected clover leaves.

Spores of *Uromyces* and *Erysiphe*, to be used in inoculations, were produced on living clover leaflets floated on a 10 per cent sucrose solution, while those of *Colletotrichum*, *Macrosporium*, and *Cicinnobolus* were produced in agar cultures. Leaflets to be inoculated were excised from disease-free plants and floated on sucrose solution or water in Syracuse watch glasses or Petri dishes.

The inoculation of the leaflets with *Erysiphe* was made by touching their upper (ventral) surfaces with another mildewed leaflet and the inoculated leaflets were floated with their lower surfaces resting on the nutrient solution and with their inoculated surfaces upward. Spores of *Cicinnobolus* were atomized in suspension on mildewed or mildew-inoculated leaf surfaces. *Uromyces* spores were dusted on the lower leaf surface, the leaflets atomized with water and floated with their upper surfaces resting on the solution. Spores of *Colletotrichum* and *Macrosporium* were atomized over the lower leaf surface in water suspension and the leaflets were floated with their upper surfaces resting on the solution.

In most of the experiments reported here the 3 leaflets of a single leaf were compared with one another, each leaflet being exposed to a different treatment. By placing each of the 3 leaflets of a number of leaves into separate dishes, 3 closely comparable units of unlimited size could be secured. Each leaflet was numbered with ink when its identity was desired. This method of comparison is similar to the "twin leaf method" of Denney (2) and will be referred to as the "triplet leaflet method." All inoculated and control leaflets were incubated at 17° to 21° C. in diffuse light, unless otherwise stated.

The observations and photographs presented usually were made about 12 days after inoculation. The incubation period, however, varied with different plants, with different parasites, and different treatments. Under optimum conditions for development, the *Colletotrichum* and *Cicinnobolus* normally sporulate about 5 days after inoculation, the *Erysiphe* in about 6 days, the *Macrosporium* in about 8 days, and the *Uromyces* in about 10 days. Injury from *Colletotrichum* and *Macrosporium* was generally evident before sporulation and the leaflets frequently died and decayed before spores were formed.

In all cases leaves from four or more plants were used in the experiments reported. The results and illustrations presented are to be considered of relative value only, as there was considerable variation in the results with different leaves and with different plants.

THE EFFECT OF DIFFERENT NUTRIENT SOLUTIONS ON THE VITALITY OF  
EXCISED CLOVER LEAFLETS

Before attempting inoculation experiments with the clover pathogens, it was necessary to determine the best nutrient solution on which to float the excised leaflets. Nonsterilized solution of commercial cane sugar (sucrose) was found most suitable for excised leaflets, thus bearing out the findings of Waters (7). The organic nutrients, sucrose, glucose, maltose, and asparagin, and the inorganic nutrients,  $\text{Mg}(\text{NO}_3)_2$ ,  $\text{CaH}_4(\text{PO}_4)_2$ , and  $\text{K}_2\text{SO}_4$  were also used alone and in different combinations as nutrients. Dextrose was better than maltose but both allowed a greater growth of contaminating fungi and bacteria than did sucrose. The mineral nutrients, as compared with distilled water, did not appreciably increase the life of the excised leaflets. Magnesium nitrate especially favored bacterial growth and  $\text{CaH}_4(\text{PO}_4)_2$  favored the growth of fungus contaminants.

Solutions, varying from 1 to 50 per cent of dissolved cane sugar, were compared as nutrients. The vigor of the excised leaflets increased with the concentration of the solution up to about 16 per cent sucrose. Healthy clover leaflets floating on distilled water usually lived 1 to 3 weeks. Leaflets on 10 per cent sucrose normally remained in good condition from 2 to 6 weeks and have been kept living for 17 weeks. The maximum concentration of sucrose on which the leaflets remained in good condition was about 32 per cent.

With age the cultures always became contaminated but contamination was not an important factor in these experiments. Cleaned but generally nonsterilized glassware was used and no attempt was made to secure aseptic leaves nor to sterilize the nutrient solutions. The cultures usually remained free of apparent contamination until the edges of some of the leaves began to decay.

CULTURES OF *UROMYCES*, *ERYSIPHE*, *MACROSPORIUM*, AND  
*COLLETOTRICHIUM*

Under field conditions these 4 parasites may be found simultaneously, but in dish cultures they show marked differences in response to the physiological condition of the leaves. In dish cultures a given leaflet generally was inoculated with only one parasite; in some cases, however, the 4 parasites were inoculated simultaneously on the same leaflet. Both methods gave essentially the same results but the former method was more satisfactory, since it was difficult to distinguish the effects of different fungi when all were inoculated on the same leaflets.

The leaflets and the condition surrounding them were modified in three ways: by floating them on solutions of varying nutrient value, by removing them at different times of the day, and by selecting leaves of different ages.

Three nutrient solutions, namely, water, 2 per cent sucrose, and 10 per cent sucrose, were found very satisfactory for determining the response differences of these pathogens to the nutritional condition of the leaflets.

### Effect of Nutrient Solution

Representative results of a test to determine the effect of the nutrient condition of the leaflets on the development of these parasitic fungi are illustrated in figure 1. Although infection resulted, leaflets inoculated

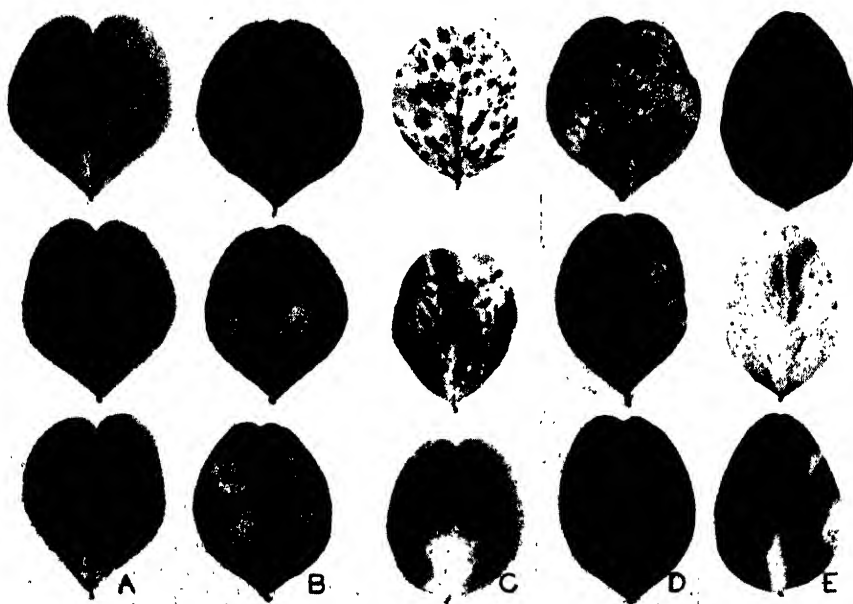


FIG. 1. Effect of sucrose nutrient solutions on the susceptibility of clover leaves to parasites. Each vertical row of leaflets is from the same leaf. Two different plants are represented. The leaflets of the upper row were floated on water, those of the center on 2 per cent sucrose, and those of the lower on 10 per cent sucrose. A. 16 days after inoculation with *Uromyces*. B. 12 days after inoculation with *Erysiphe*. The leaflets are somewhat atypical in that leaflets inoculated with mildew and floated on water normally show considerable chlorosis. C. 11 days after inoculation with *Macrosporium*. D. 14 days after inoculation with *Colletotrichum*. E. 14 days after removing from the plant but not inoculated.

with *Uromyces* showed little if any injury on any of the solutions. The optimum concentration of sucrose solution for rust sporulation was about 10 per cent; maximum, about 16 per cent. In most cases there was very little sporulation on leaflets on 2 per cent sucrose, and none on leaflets on water.

Compared with controls, leaflets inoculated with *Erysiphe* and floated



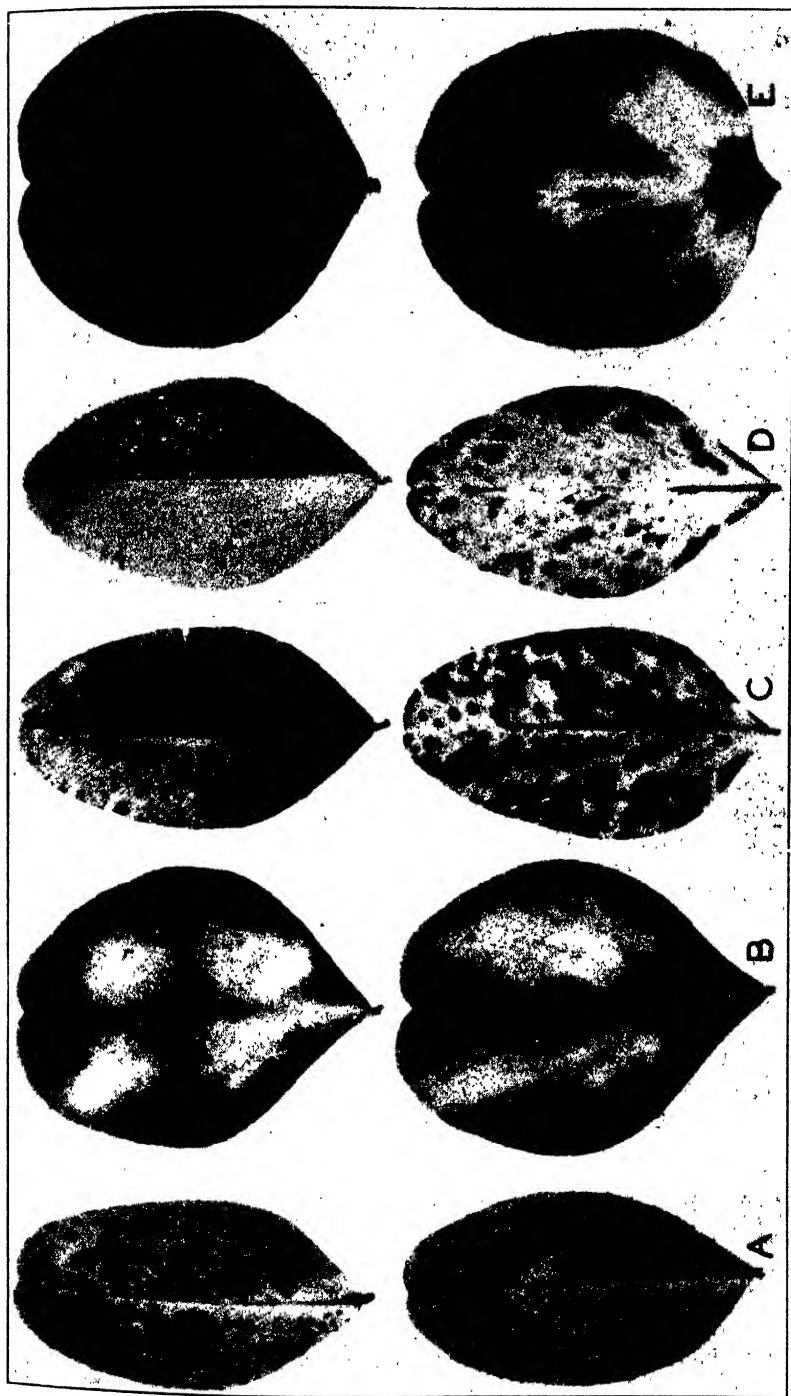


FIG. 2. Effect of the time of removing leaflets from the plant on their susceptibility to parasites. Each vertical pair of leaflets is from the same leaf. Different plants are represented. The leaflets of the upper row were removed at 5 p.m. and those of the lower row on the following morning at 7 a.m. A. 13 days after inoculation with *Uromyces*. B. 10 days after inoculation with *Erysiphe*. C. 7 days after inoculation with *Macrosporium*. D. 10 days after inoculation with *Colletotrichum*. E. 12 days after removing from plant but not inoculated. Leaflets in A and B floated on 10 per cent sucrose; in C to E on water.

on water were generally severely injured. The injury decreased as the sucrose concentration of the medium was increased up to 16 per cent. The sporulation of *Erysiphe* was greatest on leaflets floating on 16 per cent sucrose and decreased with increasing or decreasing sucrose concentrations; but the optimum range was not so clearly defined as for *Uromyces* infection.

Leaflets inoculated with *Macrosporium* were severely injured when floated on water, less so on 2 per cent sucrose, and least on 10 per cent sucrose (Fig. 1, C). *Macrosporium* sporulated very little on leaflets on water but fairly abundantly on leaflets on 2 per cent sucrose. Leaflets on 10 per cent sucrose were apparently very resistant at first but, as the leaflets aged, the fungus sporulated heavily on the dead and dying parts.

*Colletotrichum* caused the greatest injury to leaflets on water and least to leaflets on 10 per cent sucrose (Fig. 1, D). The greatest initial sporulation was coincident with the greatest injury to the leaflets, but, later, the greatest sporulation occurred on leaflets on 2 per cent sucrose. No sporulation of *Colletotrichum* was observed on leaflets on 10 per cent sucrose.

#### THE EFFECT OF THE TIME OF DAY OF REMOVING THE LEAVES FROM THE PLANT ON SUSCEPTIBILITY TO AND DAMAGE FROM INFECTION

Several workers, among them Miller and others (4), have shown that, due to photosynthesis, the carbohydrate and dry matter contents of green leaves normally increase during the day, reach a maximum in the afternoon, and decrease to a minimum during the night and early morning. Since it appears that the carbohydrate content of the leaves is a factor affecting their susceptibility to disease, as was indicated by the results with sucrose nutrient solutions, it may be reasoned that the time of day when the leaves were removed from the plant, before being inoculated in dish cultures, would affect their susceptibility to disease.

Experiments to investigate this possible relationship were performed in March and April. Two times of removal, one at about each extreme of the normal diurnal variation in the carbohydrate content of the leaves, were found most satisfactory for demonstrating the effect of time of removal of leaves on their vigor and on their resultant susceptibility to disease. Each leaflet of a leaf was removed from the plant at a different time but all were inoculated at the same time and subjected to subsequently similar conditions. One leaflet (A) of each leaf tested was removed at 5 p.m. of a clear day. The leaflets removed were placed in the dark on a nutrient solution and the plants from which they were removed were placed in the dark. Another leaflet (B) from each of the same leaves was removed from the plant the following day at 7 a.m. and also placed on nutrient solution in the dark. The growing plants were then returned to the light. The third leaflet (C)

was removed at 5 p.m. of the second day. All leaflets were inoculated simultaneously, placed in the dark, and examined after about 5 days.

For these experiments the uninoculated leaflets, the leaflets inoculated with anthracnose, and the leaflets inoculated with leaf spot were floated on water during the incubation period. Because of the poor development of mildew and rust, which resulted when the host leaflets were floated on water or on weak sucrose solution, these leaflets were placed on 10 per cent sucrose.

After several days on distilled water the noninoculated leaflets excised from the plants in the late afternoon were more vigorous than similarly treated leaflets that had been excised the following morning (Fig. 2, E).

The differences in susceptibility of leaflets removed from the plant at different times were most clearly evident on leaflets inoculated with anthracnose. Leaflets removed at 5 p.m. were more resistant than leaflets from the same leaves removed at 7 a.m. the following morning (Fig. 2, D). Similar results were secured by removing leaflets from the plant at 4 different times of the day. These leaflets were taken at 6 a.m., noon, 6 p.m., and midnight. In this comparison the leaflets taken in the late afternoon (6 p.m.) were again the most vigorous and most resistant, while those taken at 6 a.m. were the least vigorous and the most susceptible. The leaflets taken at noon and midnight were intermediate between these extremes of vigor and susceptibility, those taken at noon being slightly more vigorous and resistant than those taken at midnight.

The different times of leaflet removal affected the relative susceptibility to leaf spot in the same way as it did the susceptibility to anthracnose (Fig. 2, C), although the differences were less marked.

Noninoculated leaflets on 10 per cent sucrose generally showed indistinctly any differences in the vigor of leaflets due to the time they were removed from the plant. No distinct differences were found when such leaflets were inoculated with mildew and rust (Fig. 2, A and 2, B), although in some cases a greater sporulation of rust and mildew occurred on the leaflets removed in the late afternoon. It is probable that reducing the concentration of sucrose would have made the original differences in these leaflets more apparent when tested for susceptibility to mildew and rust.

To determine the effect of the age of clover leaves on their susceptibility to disease in dish cultures, leaves of different ages from the same plant were inoculated simultaneously with the same fungus. Young leaves that had just unfolded, leaves of medium age, and the oldest leaves available and still in good condition were selected for the comparison of the effect of the three degrees of leaf maturity. In these tests leaflets inoculated with rust or mildew were floated on 10 per cent sucrose; with leaf spot on 2 per cent sucrose, and with anthracnose on water.



FIG. 3. Effect of age on susceptibility of leaves to parasites. Each vertical row of leaflets is from the same plant, the upper from a young leaf, the center one from a leaf of medium age, and the lower one from an old leaf. A. 16 days after inoculation with *Uromyces*. B. 10 days after inoculation with *Erysiphe*. C. 9 days after inoculation with *Macrosporium*. D. 12 days after inoculation with *Colletotrichum*. E. 12 days after removal from the plant but not inoculated. Leaflets in A and B floated on 10 per cent sucrose; in C on 2 per cent sucrose; in D and E on water.

The vigor of the leaflets when floated on 10 per cent sucrose or water, as measured by their ability to remain in good condition for the period of the experiments, definitely decreased with the age of the leaves selected. Young leaves were generally more vigorous than old leaves (Fig. 3, E).

The rust development on young, medium-age, and old leaflets was about the same (Fig. 3, A). With few exceptions mildew grew more vigorously on young and medium-age leaflets than on old leaflets (Fig. 3, B).

Leaf spot and anthracnose, on the other hand, made a more luxuriant development and caused greater injury on old than on young leaflets (Fig. 3, C and D).

#### CULTURES OF *CICINNOBOLUS CESATHI* DEBARY

Excised clover leaflets in dish cultures have been found very convenient for inoculations with *Cicinnobolus*. The leaflets were first inoculated with

Erysiphe and immediately after or at a desired later interval were inoculated with *Cicinnobolus*. Pycnidia of *Cicinnobolus* were formed in the conidiophores of Erysiphe in about 5 days and were most luxuriant following inoculations on young mildew colonies. The dish culture method was successfully used for cross inoculations with different isolations of *Cicinnobolus* on different mildews.

#### USE OF ENTIRE LEAVES

In a few experiments inoculations with clover mildew were made on entire clover leaves, with the petiole in a nutrient solution in a flask and the lamina in such aerial environment as was desired. Here an accurate comparison of the value of sugar solutions and water as nutrients was not made but it was clearly shown that leaves with their petioles in a weak sugar solution lived longer and supported a more vigorous growth of mildew than leaflets with their petioles in water. However, the optimum concentration of sugar solution under these conditions was only about 2 per cent, much lower than for excised leaflets floating on the solution.

#### DISCUSSION

There are obviously certain advantages and certain disadvantages in the use of excised leaves in comparison with the use of entire plants in infection experiments. The main disadvantage, already given by Clinton and McCormick (1), is the relatively early death of excised leaves. Another disadvantage is the difficulty of relating results from experiments of this type to results under natural conditions.

Some of the advantages of the method are as follows (the first 4 of these are given by Clinton and McCormick):

1. Economy of space.
2. Economy of host material.
3. Ease and exactness of observation.
4. Reduced danger from contamination. This is especially important with wind-blown fungi, such as powdery mildews, and when physiologic forms are being studied.
5. Uniformity of experimental units. In a species such as red clover genetically uniform experimental material can be secured only by some method of vegetative propagation, and this method with excised leaflets in dish cultures is more expedient than cuttings for many purposes.
6. Ease of control and modification of environmental factors.
7. More luxuriant development and sporulation of parasitic fungi.

#### SUMMARY

Methods for the culture of a number of parasitic fungi on excised red-clover leaves are described and discussed.

Leaflets maintained in a high degree of vigor by floating on 10 per cent sucrose solution were more susceptible to *Uromyces* and *Erysiphe* and less susceptible to *Macrosporium* and *Colletotrichum* than leaflets floated on 2 per cent sucrose or on water.

Leaflets removed from the plant in the late afternoon were more vigorous and more susceptible to *Uromyces* and *Erysiphe* and less susceptible to *Macrosporium* and *Colletotrichum* than leaflets removed from the plant in the early morning.

Young excised leaflets were more vigorous and more susceptible to *Erysiphe* and less susceptible to *Macrosporium* and *Colletotrichum* than old leaflets.

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# SWEET-POTATO RING ROT CAUSED BY *PYTHIUM ULTIMUM*<sup>1</sup>

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The writings of Halsted indicate that he observed the ring rot (Fig. 1, A) of sweet potatoes in 1890 (1). In 1894, he attributed its cause to a *Fusarium* (*Nectria ipomoeae*) (2). Taubenhause, after studying the disease in 1915, concluded that *Rhizopus nigricans* is the causative organism of both the distinct ring and soft rots (6). Further studies have shown that the ring rot of sweet potatoes is caused by *Pythium ultimum* Trow. It has been shown to cause also rootlet rot and mottle necrosis, which are widely distributed in nature (3, 4). Harter and Weimer described 3 distinct types of decay of sweet potatoes found in the field resulting from *Pythium ultimum* infections. They were classed as band, marble and cheesy types. The marble and cheesy symptoms occurred during high and low soil temperature conditions, respectively (4). The results of studies on the relations of this fungus to ring-rot formation in sweet potatoes and the effect of the time of harvest on the control of ring rot are discussed in this paper.

As has been pointed out by Taubenhause, ring rot is known to cause heavy loss of sweet potatoes in both bank and house storage and to develop during the first few weeks after the sweet potatoes have been harvested and placed in storage. Mottle necrosis of sweet potatoes was reported as causing losses as high as 40 per cent in fields in New Jersey (4). Ring rot, considered only as a storage disease, caused from 1 to 20 per cent loss in 60 storage houses examined in Delaware, Maryland, and New Jersey (6). These losses, together with those resulting from *Pythium ultimum* decay of rootlets (4) and of new plants immediately after germination, gives the fungus conspicuous rank as a parasite on the sweet potato.

During the harvest period of 1925, *Pythium ultimum* was isolated from Yellow Jersey sweet potatoes in Cumberland County near Vineland, New Jersey. Sweet potatoes generally are harvested 10 to 15 days later in Atlantic and Cumberland Counties than in Gloucester County. The losses from mottle necrosis and ring rot were invariably worse in the former counties. Cold rains fell frequently during this period of delayed harvest. Since 1926 the soft-rot symptom resulting from *Pythium ultimum* infection has been observed each season on Nancy Hall and Yellow Jersey varieties and to a limited extent on Porto Rico strains in both banks and houses in North Carolina. It has been worse in late autumn after the soils accumu-

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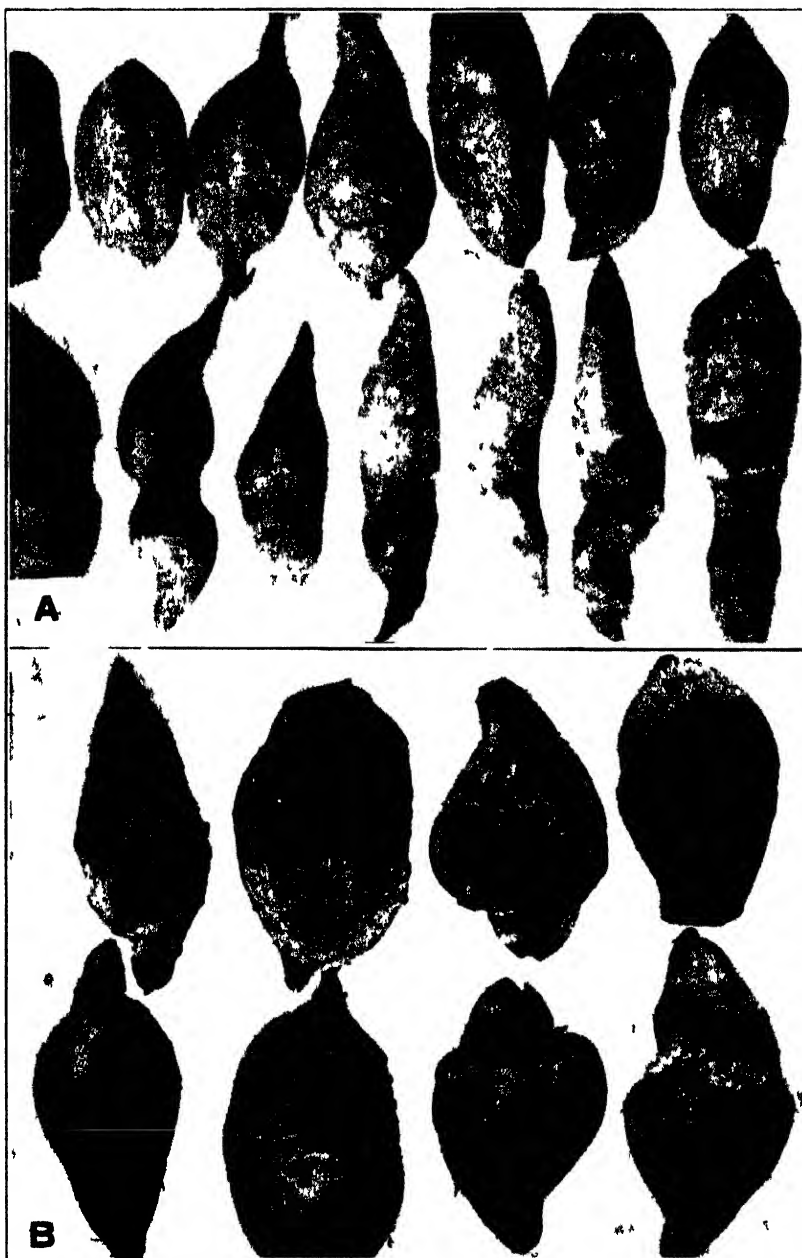


FIG 1 A Various symptoms of ring rot on Nancy Hall sweet potatoes collected in storage B Sweet potatoes with *Trichoderma*, *Fusaria*, and *Penicillia* growing externally on tissues decayed by *Pythium ultimum*



lated greater amounts of moisture and the soil temperatures became lower. Isolations were made without difficulty. Pure cultures were readily obtained from platings of tissues from recently decayed sweet potatoes on a medium composed of 250 grams of sweet potato and 12 grams of agar in a liter of water. The fungus was not isolated from the ring rot of sweet potatoes after they had been in storage 4 months. The organism has been grown on many plant decoctions and on synthetic media. Charles Drechsler identified the fungus as *Pythium ultimum* Trow (7). In recent correspondence, he stated that the diseases caused by this fungus had been attributed to *Pythium debaryanum*, even by de Bary.

For studying ring rot in the laboratory, sweet potatoes of a white strain of the Yellow Jersey variety were disinfected by dipping for 5 minutes in 1:3000 strength of mercuric chloride. Incisions one-fourth inch deep were made in the sweet potato with a sterile glass tube (Fig. 2, A) or a knife (Fig. 2, C). Inoculations from 1-day-old transfers of pure cultures and infected tissues from recently decayed sweet potatoes were used in making inoculations in the incisions. The inoculated sweet potatoes were incubated at 20° C. in quart jars containing 25 cc. of sterile water. Decay was pronounced 48 hours after inoculation with mycelium from the tubes and in 36 hours when the decayed tissues were used (Fig. 2, A and G). Rings developed rapidly. All tissues of single sweet potatoes became infected in 3 to 7 days, when left in the jars, depending on the size of the sweet potato (Fig. 2, E). The ring symptom was obtained by following the usual procedures used in storing and curing the sweet potato, since, after harvest, the sweet potato is immediately subjected to a temperature of 29 to 30° C. and good ventilation for 2 weeks.

In this study, the inoculated sweet potatoes were removed from the jars immediately after the ring was complete (Fig. 2, A) and dried at 30° C. The diseased tissues dried and shrunk rapidly in 7 days as shown on both sides of the same sweet potato (Fig. 2, B and C). Suppression of further spreading of infection was surprisingly rapid during the drying process. After drying the small sweet potatoes for 7 days, the fungus in the decayed tissues had become inactivated, since attempts to reculture it were unsuccessful.

The definite character of ring rot is easily obtained, and as many as 6 well-marked rings of decayed tissues were obtained from separate inoculations in a single sweet potato (Fig. 2, J). In these studies, 100 per cent infection was readily obtained.

The infected tissues are soft and at first of a grayish color. Extreme softness, such as that resulting from infections of *Rhizopus nigricans*, does not occur. A free liquid is, however, readily squeezed from the tissues of rapidly decaying sweet potatoes. The tissues break firmly, but leave



FIG. 2. A. Advancement of decay in sweet potato 36 hours after inoculation with a pure culture of *Pythium ultimum*. B and C. Sweet potato showing ring development on all sides after infected tissues have shrunk from loss of water as a result of pure-culture inoculation. D. End-rot and mottle-necrosis characters resulting from pure-culture inoculations with *P. ultimum*. E. Complete decay and development of white mycelium on cut surface of sweet potato incubated for 7 days in moist atmosphere. F. Black sporangia developing on surface 12 hours after sectioning sweet potato decayed by *Rhizopus nigricans*. G. Section of sweet potato showing advancement of decay 36 hours after inoculation with a pure culture of *P. ultimum*. H. Grayish and soft cheesy characters of diseased tissues and dark oxidized healthy tissues next to infected tissues 24 hours after ring rot was produced. I. Section through ring showing starch that is dry and white in cells after pectin, protein and water have been destroyed by the disease. J. Six rings produced in a single sweet potato by inoculating with *P. ultimum*. K. Type of ring produced by *R. nigricans* showing dark sporangia developing through natural openings and broken tissues of decayed sweet potatoes. L. Stringy and soft tissues of sectioned diseased sweet potato resulting from inoculating with *R. nigricans*.

ragged edges. After the infected sweet potatoes have dried for 24 hours, the decayed parts become moderately soft, somewhat comparable in character to very soft cheese (Fig. 2, H). Later, the dried tissues become loose and crumbly (Fig. 2, I). As a result of the destruction and removal of pectin from the middle lamella, and the removal of protein from the tissues, the decayed and dried parts of the potatoes are nearly white. Water is released to such an extent that the undisturbed, except separated cells full of starch, are prominently displayed (Fig. 2, I). Taubenhaus intimated that the starch was broken down, but his illustrations apparently show heavy starch deposits left in the decayed tissues after decay was stopped (6). Neither *Pythium ultimum* nor *Rhizopus nigricans* displays any definite diastatic action, even up to the time when the fungi cease to be active in the decayed tissues. Slight oxidation occurs in tissues not infected and adjacent to diseased tissues (Fig. 2, I and J). However, the acid reaction that is produced by the fungus in the tissues of the sweet potato, apparently prevents the development of the dark color due to oxidation. Changes in reaction from pH 6.5 in healthy tissues to pH 4.4 in tissues decayed by *Pythium ultimum* and to pH 4.0 in tissues decayed by *Rhizopus nigricans* were observed.

A definite end rot resulted from placing the inoculum of *Pythium ultimum* in the end of the sweet potato and it was seen that the fungus showed a slight tendency to follow the bundles, giving the mottle-necrosis symptom (Fig. 2, D). The mycelium becomes constricted at the cell wall, which it enters by force. There is no evidence of the production of cytase. The combined intercellular and intracellular activities of *Pythium ultimum* would seemingly offer sufficient explanation of the development of the ring character, since infection works from the center of infection uniformly in all directions (Fig. 2, A and G). There is no evidence that *Rhizopus nigricans* enters the cells but it shows greater activities in destroying the middle lamella than does *Pythium ultimum*. This partly accounts for greater decay of the sweet potato as a whole resulting from infection by *Rhizopus nigricans*, as compared to the constricted rings produced by *Pythium ultimum*.

In the field, infection takes place through the stem end and through feed roots on the sweet potatoes (Fig. 3, A). The rootlets are decayed in 3 days. The new plants begin to show decay in 36 hours and continue to decay rapidly (Fig. 3, B). Total decay of young plants was obtained without destroying the mother sweet potato. The mother sweet potato did decay if it became infected later and high moisture conditions were maintained. After infection had become established it continued even under drying conditions until considerable water was removed from the tissues. Temperatures of 29 to 30° C. used during the curing of sweet potatoes in storage prevented

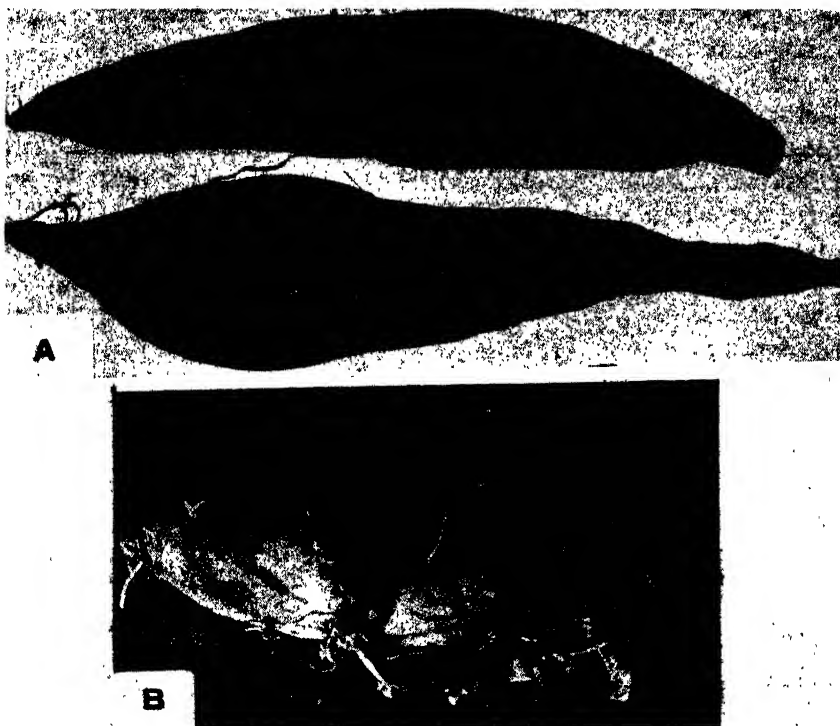


FIG. 3. A. Sweet potatoes showing infections of *Pythium ultimum* at ends and through small roots. B. Rootlets, terminal growth and leaves decayed by *P. ultimum*.

infection, even when large amounts of inoculum were inserted, indicating that infection resulting in ring rot is established in sweet potatoes before they are stored. Infection may, however, frequently occur immediately after the potatoes are stored, since high humidity persists in the various types of storage for 3 or 4 days.

Ring rot was produced readily in Nancy Hall, Triumph, Norton Yam, Red Jersey, Yellow Jersey, and Big Stem Jersey sweet potatoes. Inoculations of Porto Rico, White Yam, and Yellow Yam resulted in decay of a minor character only. String beans, beets, Irish potatoes, turnips, and radishes decayed rapidly. A copious growth of mycelium was produced on all of these plants. Carrots were slightly affected. Artichokes, onions, and parsnips did not decay, even when a heavy inoculum of freshly decayed tissues was inserted in deep punctures.

Results of studying the occurrence of infection in the field and the development of ring rot in storage and in the laboratory clearly indicate that moisture exerts a controlling influence on the growth of the causative fungus. Sweet potatoes harvested and stored by October 15 rarely show

ring rot in storage. Those harvested later, especially following cold rains, result in tremendous losses. Schmidt shows a 5-year average loss of 44.41 per cent in sweet potatoes when harvested and stored after heavy frost in comparison with 5.39 per cent when harvested and stored on October 20, before low temperatures developed in the soil and while the soil moisture was relatively low (5). His results do not indicate the specific causes of the losses, but *Pythium ultimum* was found by the writer to be the cause of sweet-potato decay in most of his tests. Nancy Hall potatoes harvested October 10, 15, 20, 30, November 10, 15, 20, and 30, 1930, showed 0, 0, 3, 8, 15, 30, 60, and 100 per cent pythium decay, respectively. Losses resulting from *Pythium ultimum* infection over a period of 3 years were irregular, since there was considerable variation in the rainfall in these seasons. The disease was produced slowly at 10° C. and more rapidly at temperatures up to 30° C.

Any fungi that produce pectinase and protease, when growing on sweet potatoes, may occasionally produce a rot that could be called ring rot. It would, however, seem unusual to find more than one fungus causing such a specific decay as that of the common ring rot. *Rhizopus nigricans* produces a somewhat disorganized type of ring rot that is definitely unlike the common ring rot (Fig. 2, K). It causes much softness, leaving the sweet potato watery and stringy. The sharply demarked borders, such as are produced by *Pythium ultimum*, were not obtained with *Rhizopus nigricans*. After making 1,000 inoculations with *Rhizopus nigricans*, using vigorous mycelium from pure cultures grown in solution and from freshly decayed tissues, it was shown that this fungus always produces sporangia through the natural openings on the surface (Fig. 2, K). The dark mycelium and skeletonized sporangia also were seen to persist on the decayed potato throughout the storage period. The sweet potatoes affected with the common ring rot rarely show the presence of *Rhizopus nigricans*, but they frequently show such semiparasitic fungi as *Fusarium* sp., *Trichoderma* sp., and *Penicillium* sp. working as saprophytes on the decayed tissues (Fig. 1, C). Sweet potatoes, decayed by *Rhizopus nigricans*, do not break without leaving stringy edges of soft watery tissues that are difficult to cut smoothly (Fig. 2, L). From 12 to 24 hours after sections are made from freshly decayed parts caused by *Rhizopus nigricans*, black sporangia develop in great masses on the cut surface (Fig. 2, F). A white mycelium develops on sections made through sweet potatoes decayed by *Pythium ultimum* (Fig. 2, E). In all respects the symptoms of the common ring rot obtained as a result of pure-culture inoculations with *Pythium ultimum* are exactly like those found in storage and known to develop soon after the potatoes are stored. It is the only time that the disease seems capable of occurring in storage, since the ability of *Pythium ultimum* to perpetuate itself by means of sporangiospores is wanting.

## SUMMARY

*Pythium ultimum* is found to cause ring rot of sweet potatoes. Infection occurs in the field. The greater losses occur after the potatoes are stored, but soft rot in the field is common.

The causal fungus was readily isolated from sweet potatoes in the process of decay. It grows well on a variety of culture media, including sweet-potato decoctions.

Ring rot was produced with the fungus after insertion of the latter into the cortex. Decay was pronounced in 36 to 48 hours after inoculating with an active culture. When the atmosphere was saturated and the temperature maintained at 20° C. in the incubation chamber, 6 well-developed rings were produced on a single potato showing the definite restricted spreading activities of the fungus.

The infection was controlled by harvesting sweet potatoes before extended wet soils favored growth of the fungus in the soil.

*Rhizopus nigricans*, after many trials, failed to produce the common ring rot.

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# FUNGICIDAL EFFECT ON *SCLEROTIUM ROLFSSII* OF SOME COMPOUNDS IN AQUEOUS SOLUTION AND IN THE GASEOUS STATE<sup>1</sup>

J. OSERKOWSKY<sup>2</sup>

(Accepted for publication September 12, 1933)

## THE EFFECT OF FUNGICIDAL VAPORS ON MYCELIUM AND SCLEROTIA

In an attempt to devise a means for the control of *Sclerotium rolfssii*, the fungicidal effect of vapors of various compounds was tested. For the study of the effect of vapors on the mycelium, the following technique was adopted. A small vial was placed upright in a stender dish and melted agar was poured into the dish. After sterilization and cooling, the vial was held in place by the hardened agar. An excess of the volatile fungicide to be tested was placed in the vial and a small block of agar supporting a fresh mycelial growth of the fungus was transferred to the surface of the agar in the stender dish (Fig. 1). The stender dish was placed in an incubator at 29°–

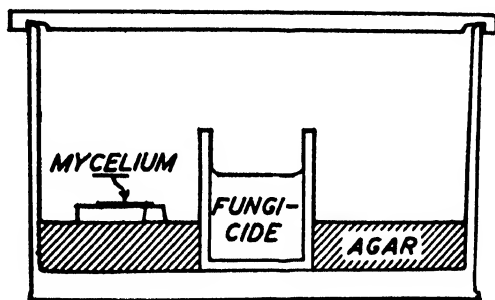


FIG. 1. Schematic drawing of stender dish and arrangement used for testing the fungicidal action of vapors on mycelium of *Sclerotium rolfssii*.

30° C. After 2 to 5 days the mycelial growth in the stender dishes containing the fungicidal vapors was compared with that made by control cultures treated alike in every respect, except that the stender dishes did not contain a fungicide. If no growth was made by the mycelium subjected to the fungicidal vapors, it was transferred to a test-tube containing fresh agar medium free of fungicidal vapors and incubated at 29° to 30° C. for 5 to 22 days: if no growth took place after this period, the fungicidal vapor was considered "lethal."

The vapors of naphthalene and its Cl and Br derivatives were found to

<sup>1</sup> Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

<sup>2</sup> It is with appreciation that the writer acknowledges the suggestions and assistance offered him throughout the work by Professors Max W. Gardner and Ralph E. Smith.

completely inhibit mycelial growth. Naphthalene had no lethal effect whatsoever, while  $\alpha$ -chlor and  $\alpha$ -brom-naphthalene were lethal to a substantial fraction of the number of mycelial inocula used. The vapor tension of naphthalene at 30° C. is 0.09 mm. Hg, that of  $\alpha$ -monochlor and  $\alpha$ -monobrom-naphthalene is approximately 0.03 mm. It is to be inferred from this that the substitution of either Cl or Br for an H atom in the  $\alpha$  position in the naphthalene molecule resulted in an enhanced toxicity to the mycelium of *Sclerotium rolfsii*.

Benzene and all its derivatives listed in table 1 were shown to be lethal to the mycelium. The vapor pressure of these compounds at 29° to 30° C. is considerably above that of naphthalene and its halogen derivatives referred to above. It is doubtful whether the benzene derivatives mentioned in table 1 would prove lethal or even growth-inhibitive at vapor pressures corresponding to those of naphthalene and its two halogen derivatives.

TABLE 1.—*The effect of saturated vapors of various substances at 29° to 30° C. on mycelial growth of Sclerotium rolfsii*

Vapors tested	Days of exposure	Number of cultures exposed	Number of cultures showing growth at the end of exposure	Number of cultures showing growth after transfer to fresh agar media
Paraffin oil, yellow .....	5	13	13	.....
Ethyl laurate .....	3	6	6	.....
Lead naphthanate .....	3	17	17	.....
Arsenic trichloride .....	3	15	0	...
Trioxymethylene .....	3	46	0	0
Naphthalene .....	3	22	0	22
$\alpha$ -monochloronaphthalene .....	3	35	0	26
$\alpha$ -monobromnaphthalene .....	3	40	0	21
Benzene .....	2	26	0	0
Toluene .....	4	27	0	0
Xylene .....	3	24	0	0
o-dichlorobenzene .....	3	50	0	2
m-dichlorobenzene .....	2	15	0	0
m-dichlorobenzene .....	3	19	0	0
p-dichlorobenzene .....	3	20	0	0
Nitrobenzene .....	3	22	0	0

The inhibitory and perhaps lethal effect of  $\text{AsCl}_3$  vapors may be due either to  $\text{AsCl}_3$  as such or to  $\text{HCl}$  and  $\text{H}_3\text{AsO}_3$ , which are readily formed in the presence of moisture.

When the effect of vapors on sclerotia was investigated the following procedure was adopted. The sclerotia were placed on the bottom of a weigh-



ing glass equipped with a ground-glass cover and containing a small vial of the substance to be tested. The whole was incubated for 3 days at 25° C. The sclerotia were then removed from the weighing glass, planted on agar media, and incubated at 29° to 30° C. The results are recorded in table 2. The data contained in this table were attained with dry sclerotia, the effect of the vapors on moist sclerotia may be different.

Since the containers used were small and closed during exposure of the fungus to the vapors of the fungicides it is safe to assume that the concen-

TABLE 2.—*Effect of exposure of sclerotia of Sclerotium rolfsii to vapors of various compounds for three days at 25° C.*

Compound	Number sclerotia tested	Percent- age of sclerotia germi- nated	Compound	Number of sclero- tia tested	Percentage germina- tion of sclerotia
Benzene	85	0	Aniline	82	94
Toluene	61	0			
Ethylbenzene	38	0			
Xylene	69	0	Mercury-di-p-tolyl	238	96
n-propylbenzene	61	0			
n-butylbenzene	49	78			
Tertiary amylbenzene	59	100	Naphthalene	62	98
			α-monobromonaph- thalene	51	92
Chlorobenzene	46	0			
o-dichlorobenzene	69	0	Chloroform	59	0
m-dichlorobenzene	113	0	Carbon tetrachloride	80	0
p-dichlorobenzene	47	100			
1, 3, 5, Trichloroben- zene	63	100	Trichlorethylene	97	96
1, 2, 4, Trichloroben- zene	56	0	S-Tetrachlorethane	78	96
1, 2, 4, 5, Tetrachloro- benzene	53	93			
Hexachlorobenzene	68	100	Pentachlorethane (Tech)	102	100
o-chlorotoluene	40	100	Chloropicrin	128	100
m-chlorotoluene	110	94			
p-chlorotoluene	74	99	Bromoform	90	18
			Bromopicrin	90	0
Bromobenzene	88	100			
o-bromotoluene	118	89	Iodine	47	0
p-bromotoluene	54	100	Iodoform	79	100
Nitrobenzene	32	0			
m-dinitrobenzene	84	100	“Oronite crystaloil”	83	100
p-dinitrobenzene	64	100	“Chlorox”	91	100
1, 3, 5, Trinitrobenzene	87	100	Carbon bisulphide	25	0
m-nitrotoluene	95	100	Trioxymethylene	37	0
o-nitrotoluene	115	96			
			Checks	956	97
2-5 dichloronitroben- zene	356	94			
3-4 dichloronitroben- zene	327	92			

tration of the vapors in the containers was close to the vapor tension of the substances investigated at 25° and 30° C., respectively. For most substances used in this study no data are available regarding their vapor tension at these temperatures; hence, no general basis of comparison of the relative toxicity of these compounds is available. In a few instances, however, where the vapor tension at 25° C. could be obtained, either directly from tables or by graphical extrapolation and intrapolation, it was possible to compare toxicity and chemical structure (Table 3). Thus it was found

TABLE 3.—*Chemical composition, vapor tension and effect of vapor on sclerotia of Sclerotium rolfsii*

Compound	Formula	Vapor tension at 25° C. mm. Hg.	Effect on sclerotia
Nitrobenzene	$C_6H_5NO_2$	0.41	lethal
Aniline	$C_6H_5NH_2$	0.59	not lethal
Bromobenzene	$C_6H_5Br$	5.05	not lethal
p-dichlorobenzene	$p-C_6H_4Cl_2$	0.86	not lethal
Bromopierin	$CBr_3NO_2$	<15	lethal
Chloropierin	$CCl_3NO_2$	18.3	not lethal

that the substitution of an  $NO_2$  group in the benzene ring resulted in a greater toxicity than the substitution of one  $NH_2$  or one Br, or 2Cl atoms in the para position. Similarly, the substitution of Br for Cl in chloropierin resulted in enhanced toxicity. It was not possible to arrive at the vapor tension of bromopierin at 25° C. At 85° to 86° C. its vapor tension is 15 mm. It must be, therefore, substantially lower at 25° C. Iodine, with a vapor tension of 0.3 mm. at 25° C., was lethal to sclerotia of *Sclerotium rolfsii*, and experimental evidence indicates that it is lethal even at a substantially lower vapor pressure.

#### THE EFFECT OF AQUEOUS SOLUTIONS OF VARIOUS SUBSTANCES ON SCLEROTIA

Sclerotia were put in small cheesecloth bags and immersed in solutions of various substances for several days at 25° to 26° C.; then each bag was passed successively through 5 flasks, containing sterilized distilled water, to remove as much as possible of the fungicidal substance. The sclerotia were then planted on agar media and incubated at 29° to 30° C. In a few instances the sclerotia were dipped in 0.1 per cent  $HgCl_2$  for 80 to 135 seconds after removal from the fungicidal solution and previous to passing through the sterilized-water flasks for the purpose of surface sterilization. Although the  $HgCl_2$  treatment may have decreased somewhat the number of sclerotia germinating, it did not materially affect the results. Some of the solutions seemed to cause a decrease in percentage of germinating sclerotia, but in view of the wide variation in the percentage of germination

in duplicate treatments, it is doubtful whether any significance can be attached to these low germination values. It was deemed advisable, therefore, to omit the percentage of germination from the following data and to divide the substances into two groups, namely, those that completely inhibited germination, and those that did not inhibit it completely. To the first group belong Merthiolate (33<sup>1</sup>, 1), Hexylresorcinol<sup>2</sup> (1000, 1) and o-chlorophenol (1000, 5). The following substances are included in the second group: p-chlorophenol (500, 3), o-chlorophenol (1000, 1), 2-chloro-2-hydroxytoluene (200, 3), picric acid (500, 4), sodium salicylate (350, 3), paraldehyde (1000, 3) saturated solution of p-nitrochloro-benzene (3 days), potassium bromide (300, 3), Na<sub>2</sub>S · 9H<sub>2</sub>O adjusted to pH 6.4 (1750, 4), sodium chlorate (1000, 3), sodium sulphite (500, 3), mercurochrome (195, 3), lead nitrate (185, 1) and CuSO<sub>4</sub> · 5H<sub>2</sub>O (200, 3).

With few exceptions the concentrations tried corresponded to what were considered to be the maximum concentrations compatible with practical use. It is unfortunate that precisely the three treatments that completely inhibited germination of sclerotia, *i.e.*, killed the sclerotia, cannot be used for practical purposes because of their prohibitive cost, even if they should prove as effective in soil as they are *in vitro*.

#### SUMMARY

Mycelium of *Sclerotium rofsii* was not killed, but its growth was inhibited during 2 to 4 days' exposure to saturated vapors at 29° to 30° C. of naphthalene, while the vapors of  $\alpha$ -monochlor-naphthalene and  $\alpha$ -mono-brom-naphthalene inhibited growth of mycelium and were lethal to some of the cultures.

The mycelium was killed by the saturated vapors of trioxymethylene, benzene, toluene, xylene, nitrobenzene and ortho, meta and para dichlorobenzene.

Sclerotia were killed by three days' exposure at 25° to 26° C. to the saturated vapors of benzene, toluene, xylene, ethylbenzene, n-propylbenzene, chlorobenzene, ortho and meta dichlorobenzene, 1, 2, 4 trichlorobenzene, nitrobenzene, chloroform, carbon tetrachloride, bromopicrin, iodine, carbon bisulphide, and trioxymethylene.

Sclerotia were killed also after immersion for one day in 1:33 dilution of Merthiolate, and in 0.1 per cent solution of hexylresorcinol, and for 5 days in 0.1 per cent of o-chlorophenol.

Substitution of an NO<sub>2</sub> radical in the benzene ring resulted in a greater toxicity than the substitution of either NH<sub>2</sub>, Br, or 2 Cl atoms in the para position. Substitution of Br for Cl in chloropicrin enhanced the toxicity.

<sup>1</sup> 1:33 dilution of the commercial product "Merthiolate."

<sup>2</sup> In the following list the first number in the parenthesis refers to the concentration of the substance in parts per million by weight, the second number indicates the number of days the sclerotia were immersed in the solution.

# CUCURBIT MOSAIC TRANSMITTED BY MUSKMELON SEED<sup>1</sup>

JAMES B. KENDRICK<sup>2</sup>

(Accepted for publication October 3, 1933)

In November, 1932, a single case of mosaic occurred in 170 seedling Persian melons growing in sterile soil in the greenhouse, at Davis, California. The spontaneous occurrence of a single mosaic plant in a large number of seedlings strongly suggested the seed as the source of the disease.

According to Doolittle and others, the cultivated species of cucurbits rarely, if ever, transmit mosaic through the seed. Doolittle and Walker<sup>3</sup> report one case of apparent seedling infection in approximately 22,000 cucumber plants and negative results in less extensive trials with muskmelon, squash, and pumpkin seed harvested from mosaic plants. It has been shown, however, that seed of the wild cucumber, *Micrampelis lobata*, is capable of transmitting mosaic.<sup>4</sup>

In order to determine whether or not the mosaic virus might be transmitted through muskmelon seed, 23  $\frac{1}{4}$ -lb. packets of 12 commercial varieties of muskmelon seed were secured from 3 different seedsmen. On March 14, 1933, seed from 14 packets, representing 11 varieties, were used to plant flats of steam-sterilized soil that were placed on a greenhouse bench. Repeated fumigation to keep out insects and the absence of cucurbit mosaic in the greenhouse precluded the possibility of introduction of the virus from sources other than the seed.

Two weeks after planting, the young plants were developing the first foliage leaves, and evidence of mosaic was present on such leaves of three plants in a flat of Honey Dew melons grown from seed packet number 1 (Fig. 1). On April 8 all plants were examined critically for mosaic symptoms, and one additional mosaic plant was found in a flat of plants from Persian seed packet number 1. The totals are presented in table 1.

As a further check on the above results, on April 11, seed from 16 packets representing 7 varieties, was planted in the same flats of soil without reesterilization. In 16 days, the plants were developing their second foliage leaves and were carefully examined for evidence of mosaic. It was found that 15 of the 268 plants from Honey Dew, seed packet number 1, and one

<sup>1</sup> Contribution from the Division of Plant Pathology, Branch of the College of Agriculture, Davis, California.

<sup>2</sup> The writer wishes to express his appreciation to Professors Ralph E. Smith and Max W. Gardner for helpful advice and criticism.

<sup>3</sup> Doolittle, S. P., and M. N. Walker. Further studies on the overwintering and dissemination of cucurbit mosaic. Jour. Agr. Res. 31: 1-58. 1925.

<sup>4</sup> Doolittle, S. P., and W. W. Gilbert. Seed transmission of cucurbit mosaic by the wild cucumber. Phytopath. 9: 326-327. 1919.



FIG. 1. A. Mosaic symptoms on a seedling Honey Dew muskmelon plant grown from commercial seed. Note the irregular dark green raised areas on the lighter green background of the first foliage leaf and the large blister-like areas on the somewhat elongated second foliage leaf. The third and fourth foliage leaves show extreme elongation and a slight upward curling of the edges. B. The third foliage leaf detached from the plant.

plant from Honey Dew, seed packet number 4, showed mosaic, while no mosaic was found in plants from the seed of the other 14 packets (Table 1).

In order to preclude all possibility of an outside source of the seedling mosaic, the flats of soil were again steam-sterilized, planted with seed from the 3 packets that had previously yielded evidence of seed transmission, and immediately covered with insect-proof cages. The cages were removed 20 days after planting and the plants examined for mosaic symptoms. There were 7 cases of typical mosaic in 513 plants from Honey Dew, seed packet number 1, while 332 plants from Honey Dew, seed packet number 4, and 399 plants from Persian, seed packet number 1, were all healthy (Table 1).

Table 1 shows that in the 1,171 plants grown from Honey Dew, seed packet number 1, 25, or 2.13 per cent, were mosaic. In the 656 plants from Honey Dew, seed packet number 4, 1, or 0.15 per cent, was mosaic and in the 1,061 plants from Persian, seed packet number 1, 1, or 0.094 per cent, was mosaic. No mosaic occurred in the plants grown from the other 20 seed packets. Out of the total of 11,519 plants grown from the 23 packets of muskmelon seed, 27, or 0.23 per cent, were mosaic.

The mosaic was successfully transferred by artificial means from the mosaic Honey Dew seedlings to the Casaba, Hale's Best, Honey Ball, Honey

TABLE 1.—*Summary of greenhouse trials with 23 packets, representing 12 varieties, of commercial muskmelon seed under controlled conditions for seed transmission of cucurbit mosaic*

Variety and packets	Trial, March 14 to April 8		Trial, April 11 to April 27		Trial, May 9 to May 29		Totals	
	Number plants	Number mosaic	Number plants	Number mosaic	Number plants	Number mosaic	Number plants	Number mosaic
Burrell Gem	371	0					371	0
Casaba			452	0			452	0
"	256	0	542	0			798	0
Hale's Best	633	0					633	0
"	205	0					205	0
"								
"			388	0			388	0
"			493	0			493	0
Honey Ball			351	0			351	0
"			253	0			253	0
"			197	0			197	0
"			268	15	513	7	1171	25
Honey Dew	390	3	413	0			684	0
"	271	0	264	0			264	0
"			324	1	332	0	656	1
"			420	0			814	0
Honey Rock	394	0					334	0
Lake Champlain	334	0					335	0
Osage	335	0					421	0
Pearl Pink Meat	421	0					402	0
Perfecto Improved	402	0					1061	1
Persian	261	1	401	0	399	0	281	0
"			362	0			362	0
"			241	0			593	0
Tip Top	352	0						

\* Where more than one packet of a variety was tested the packets were numbered to designate the source of the seed.

Dew, and Tip Top varieties of muskmelons and to the Early White Bush Scallop, Giant Summer Crookneck, and Italian varieties of squash. In each case 100 per cent infection was secured, and symptoms comparable in every way to the disease on the seedling Honey Dew plants appeared in 10 to 12 days.

#### SUMMARY

The above tests indicate that cucurbit mosaic can be transmitted with muskmelon seed. In controlled experiments with 23 packets, representing 12 varieties of commercial muskmelon seed, a small percentage of successful seed transmission was secured from 3 of the 23 packets.

## PHYTOPATHOLOGICAL NOTES

*Reaction of Alfalfa Varieties to Stem Blight.*<sup>1</sup>—Stem blight<sup>2</sup> is an important disease of alfalfa in Utah; it is also a factor that will function prominently in determining the relative value of any alfalfa variety to be introduced into the State or possibly into any part of the intermountain region of the United States. During the crop season of 1933 the writer had an excellent opportunity to study the reaction of a number of alfalfa varieties to alfalfa stem blight under conditions that appear to add special significance to the information obtained. The important relation of this information to the possible value and general adaptability of recommended wilt-resistant varieties of alfalfa seems to the writer to justify an early statement, even though this information presented is based upon only a year's observations.

### Source of Data and Method of Collection

The varieties of alfalfa listed in figure 1 were planted in 1932 in plots 18 × 21.5 feet and so randomized as to give effective distribution of related varieties over the entire field.<sup>3</sup> A number of varieties were replicated 3 times, others twice, and a few were grown in single plots. These plantings were made primarily for the purpose of testing these varieties to determine their relation to bacterial wilt.

The spring of 1933 was especially cold and wet. Low temperatures prevailed throughout the greater part of May, with killing frosts on May 9 and 11. This resulted in severe frost injury to nearly all varieties, which at this time ranged in height from 4 to 8 inches. Following this injury, alfalfa stem blight developed throughout the entire field, resulting in extremely severe damage, particularly to certain varieties. The disease was so severe as to reduce yields in these varieties in the first crop as much as 40 to 50 per cent. Final observations on the degree of severity of the disease in the various varieties were made on June 16 and 20.

In making observations a wire hoop, 18 inches in diameter, was placed at random in various plots. The stems thus encircled were cut close to the ground, collected, and classified according to the amount of alfalfa stem

<sup>1</sup> Contribution from Department of Botany and Plant Pathology, Utah Agricultural Experiment Station. Publication authorized by the Director, March 13, 1934.

<sup>2</sup> The specific etiology of the disease here designated as stem blight was not determined. While Sackett's work on etiology has been generally accepted, it is important to note that the author has isolated a *Phoma* species with considerable uniformity. This fact is especially significant in view of the recent work of Johnson and Valleau in Kentucky on black stem of alfalfa and other legumes.

<sup>3</sup> Data were collected from wilt-resistant varieties planted cooperatively by R. J. Evans, Agronomist, and the writer.



blight with which they were affected. Three loops, collected from each plot, had an average of 160 stems per loop, or approximately 450 to 480 stems per plot. The total number of stems for each variety used was 1, 2, or 3 times the average of 450 per plot, dependent upon the number of replications of the variety.

#### Derivation of a Coefficient of Varietal Susceptibility<sup>4</sup>

All diseased stems collected were segregated into 6 classes, dependent upon the degree of severity exhibited: (1) Very light, (2) light, (3) average, (4) severe, (5) very severe, and (6) dead. These classes were then assigned consecutive numbers from 1 to 6, inclusive, each consecutive number corresponding to the relative increased degree of severity: No 1 indicated least and No. 6 the greatest severity.

The number of stems in each diseased class was multiplied by the index number for the class; the sum total of numbers thus derived was divided by the total of diseased and disease-free stems collected from each plant. This figure, which represented an average of figures derived from the various replications, was then accepted as the coefficient of susceptibility for each variety of alfalfa studied. Coefficients for the various alfalfas are shown in figure 1.

TABLE 1.—*The comparative severity of alfalfa stem blight in typical plots of Ladak and Turkestan alfalfa in Salt Lake County, Utah*

Degree of severity	Variety	
	Turkestan	Ladak
<i>Class</i>	<i>No. stems</i>	<i>No. stems</i>
Free .....	16	279
Very light .....	116	21
Light .....	120	3
Medium .....	74	0
Severe .....	14	0
Very severe .....	1	0
Dead .....	2	0

The severity of stem blight in so large a number of varieties as were tested suggests the desirability of combining both stem blight and wilt resistance in all alfalfa varieties to be grown under Utah conditions. This will be especially true for varieties to be grown in the higher valleys in the State, as well as throughout the intermountain section as a whole.

<sup>4</sup> The word "susceptibility" is used here primarily in the sense of degree of severity of the disease exhibited in the different varieties. No specific resistance in those varieties that remained free or relatively free from the disease is definitely implied.

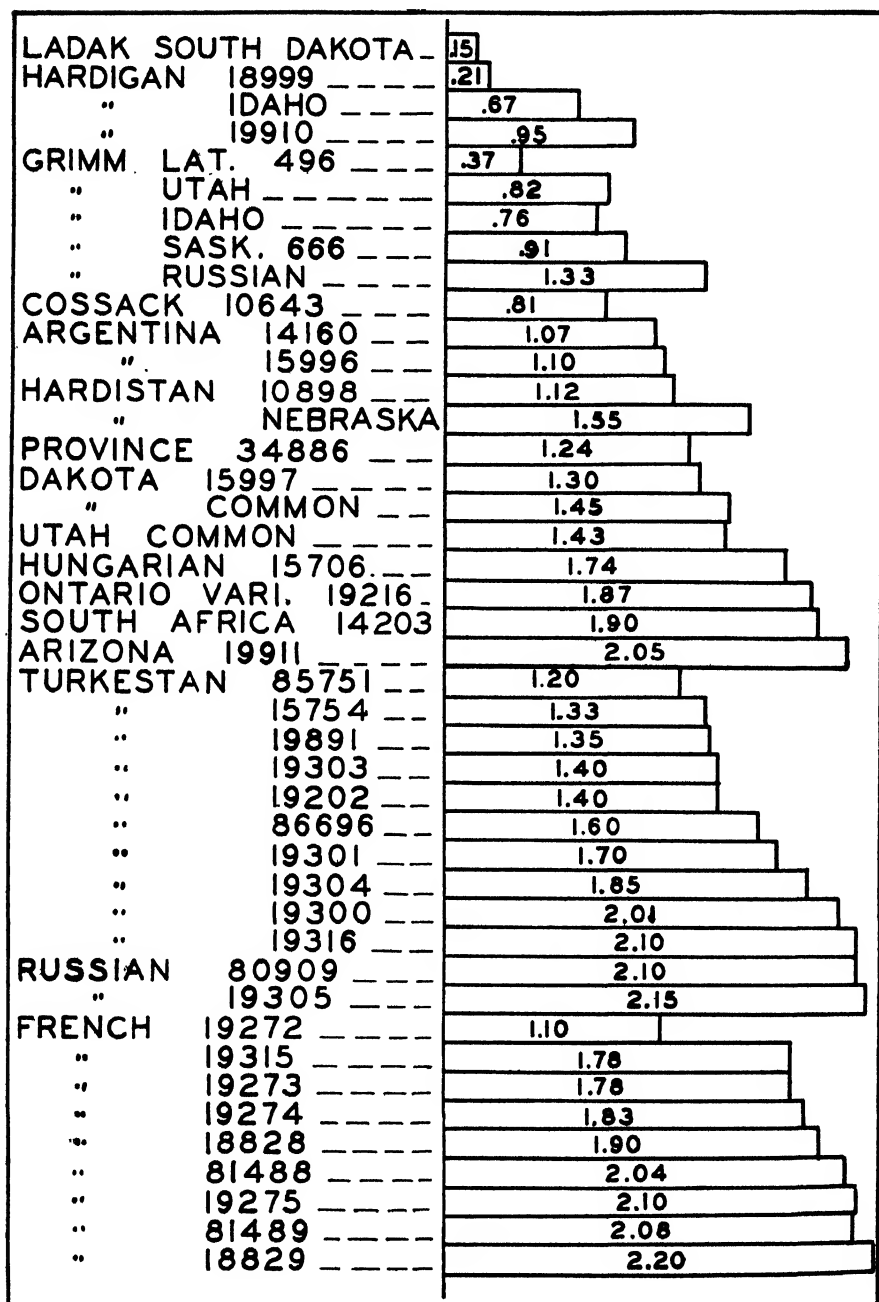


FIG. 1. Coefficient of susceptibility of alfalfa varieties to stem blight, based upon one year's observations only.

The significance of one year's (1933) observations as to the degree of true resistance exhibited in various alfalfas to stem blight, as well as the nature of resistance involved remains to be determined by further detailed observations and by extensive artificial inoculation studies. B. L. RICHARDS, Utah Agricultural Experiment Station, Logan, Utah.

*Black Spot of Germinating Pea Seed.*<sup>1</sup>—During the past several years a peculiar black spotting of germinating pea and bean seed has been noted in the seed-testing laboratory. The trouble has become more noticeable each year until, in 1933, blackened seeds were found in all of several hundred samples of peas, beans, and Lima beans representing the commercially important varieties. Lots from every principal seed-growing region, as well as from local acreages, were spotted abundantly.

The spots are dull olivaceous to sooty black, and appear most strikingly at the termination of the usual 6-day germination period. They are particularly evident on white peas and beans, but can be detected readily on green pea seed, less so on red, and only with difficulty on brown and black seeds. Samples germinated at 25° C. usually contain more spotted seeds than do samples germinated at 20° or 30° C. In some cases a single spot may seem to envelope the entire surface of a seed, and more frequently, as many as 50 to 100 minute spots may be present; but most of the contaminated seeds are partially blackened by from 3 to 10 spots, varying from 1 to 5 mm. in diameter (Fig. 1, A). The spots appear to be confined to

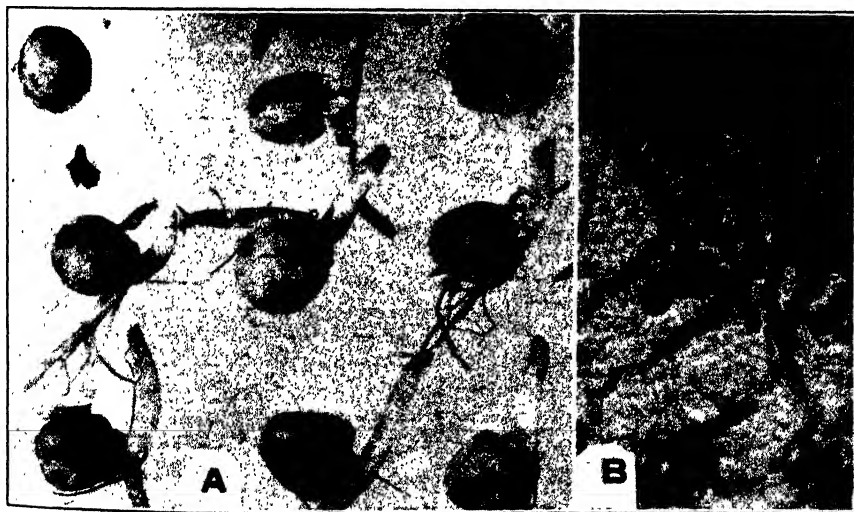


FIG. 1. *Dematium pullulans* (f) on peas. A. Mycelial masses on germinating seeds. B. Photomicrograph of hyphae on a seed coat.  $\times 220$ .

<sup>1</sup> Approved for publication by the Director of New York State Agricultural Experiment Station as Journal Paper No. 34.

the seed coats, since smearing infected tissues over the cotyledons has never resulted in the formation of the typical black areas.

The blackened areas are superficial masses of fungus hyphae. The mycelium consists of long many-septate hyphae that in most cases consist entirely of black or dark-brown cells but in some instances of hyaline and alternate hyaline and black cells. The hyphae vary greatly in diameter,  $4\ \mu$  to  $16\ \mu$ , and the diameter of a single hyphae is rarely uniform over any considerable length. The septa may occur so close together that the width of a cell exceeds the length, although (Fig. 1, B) the length is usually  $1\frac{1}{2}$  to 2 times the width. In fully developed hyphae constrictions at the septa create the impression of long strands of catenulate spores, not unlike chlamydospores of certain species of *Fusarium* or of so-called spores of *Torula* species. Branching occurs very irregularly and appears to be due to budded outgrowths from the lateral walls of the cells. Short-ovate to cylindric, hyaline spores,  $3.5\text{--}6.3\ \mu \times 6.5\text{--}12.5\ \mu$ , develop from the walls of the hyphae on short sterigmata. They are present infrequently on the seed coats but form rapidly when infected tissue is placed in water.

Since no perfect stage has been found, the fungus is regarded as a member of the family Dematiaceae. It has not been assigned definitely to any species but its appearance and habits of growth suggest the fungus known as *Dematium pullulans* de By. et Löw.<sup>2</sup>

The extremely common occurrence of the fungus on pea and bean samples indicates that it is distributed easily in the field or at the growing stations or else it is spread from sample to sample in the germination room. The latter thought was considered highly probable, since each sample in a large shipment of peas is placed on wet paper toweling with a counting board. The board becomes moist through contact with the damp towels so that any adhering spores might easily become distributed to other samples. It has been noticed that samples placed on the towels by hand always contain fewer spotted seeds than do those handled by the counter method. Comparative tests employing the 2 methods have demonstrated definitely that the counter may distribute the fungus to other samples, but since most samples are infested when they arrive at the laboratory, this form of contamination is of negligible importance. Green peas collected direct from the pods with sterilized forceps failed to show any spots, thus indicating that infestation must occur after the seeds are harvested and threshed. Further opportunity for contamination occurs in the grading, bagging, and elevation operations. Ample proof of this possibility was evidenced when green peas, taken direct from clean pods and then mixed with dried spotted tissues, developed the typical black areas.

<sup>2</sup> Drs. C. L. Shear, F. A. Wolf, F. L. Stevens and A. E. Jenkins kindly examined cultures for which thanks are gratefully given.

Although all indications negated an active pathogenicity, experiments were outlined to determine definitely this point. Pea and bean plants of 49 varieties were inoculated at various ages and by different means. Water and decoction suspensions, agar cultures, and infected tissues were employed. Spraying or rubbing the fungus on the foliage, pods, or blossoms, or puncturing it into the stems or pods gave negative results. Attempts to develop the fungus on seedlings in the germinators also were unsuccessful.

The writer would welcome any information from pathologists or seed-laboratory workers who may have observed this black spotting on germinating peas.—WILLARD CROSIER, New York State Agricultural Experiment Station, Geneva, New York.

*A Modification in Incubator Construction.*—An incubator of large capacity being required and the necessary funds for its purchase unavailable, the incubator herein described was designed and built at a cost of about one-third that of standard commercial types. It has a volume of 18.5 cubic feet and a shelf space of 4030 square inches. The interior is 42 inches high by 30 inches wide by 26 inches deep, and is provided with 5 heavy wire shelves. The construction of the walls is shown in Figure 1, A. The heat insulation of each wall consists of  $\frac{1}{8}$  inch pressboard,  $\frac{1}{4}$  inch air space,  $\frac{1}{2}$  inch insulation board,  $\frac{1}{4}$  inch air space and  $\frac{1}{2}$  inch insulation board from inside to outside in the order given.

Air circulation is provided through the arrangement shown in figure 1, B. By means of the false top and side walls, a 1-inch space is left between the incubator interior and the external walls through which warm air is forced by a blower. This warm air first passes through the top-wall air space and then down the side-wall air spaces to emerge through 1-inch slots at the bottom of the compartment. Passing up through the incubator interior, the air enters the control box on top where it is warmed, if necessary, before again entering the blower (Sirocco 00). This arrangement would appear to have several advantages over conventional arrangements. The air is circulated and all of it passes over the thermostat instead of attempting to maintain temperature uniformity through air circulation by temperature difference in the incubator interior. Temperature differences in the compartment are avoided also by placing the heaters outside, *i.e.*, in the control box. The warm air, before entering the interior, warms it by conduction from the top and side air spaces. Lastly, heat can be lost from the interior only through 3 (back, front, and bottom) of its sides.

The interior is free of all control equipment and electrical parts, excepting a 10-watt lamp to furnish light and the thermostat in the air outlet. These are located on top of the incubator. The heater (75-, 150-, 300-watt) is in the interior of the control box at the blower inlet while the switches, relay, and indicator lamp are mounted on the front of the control box.

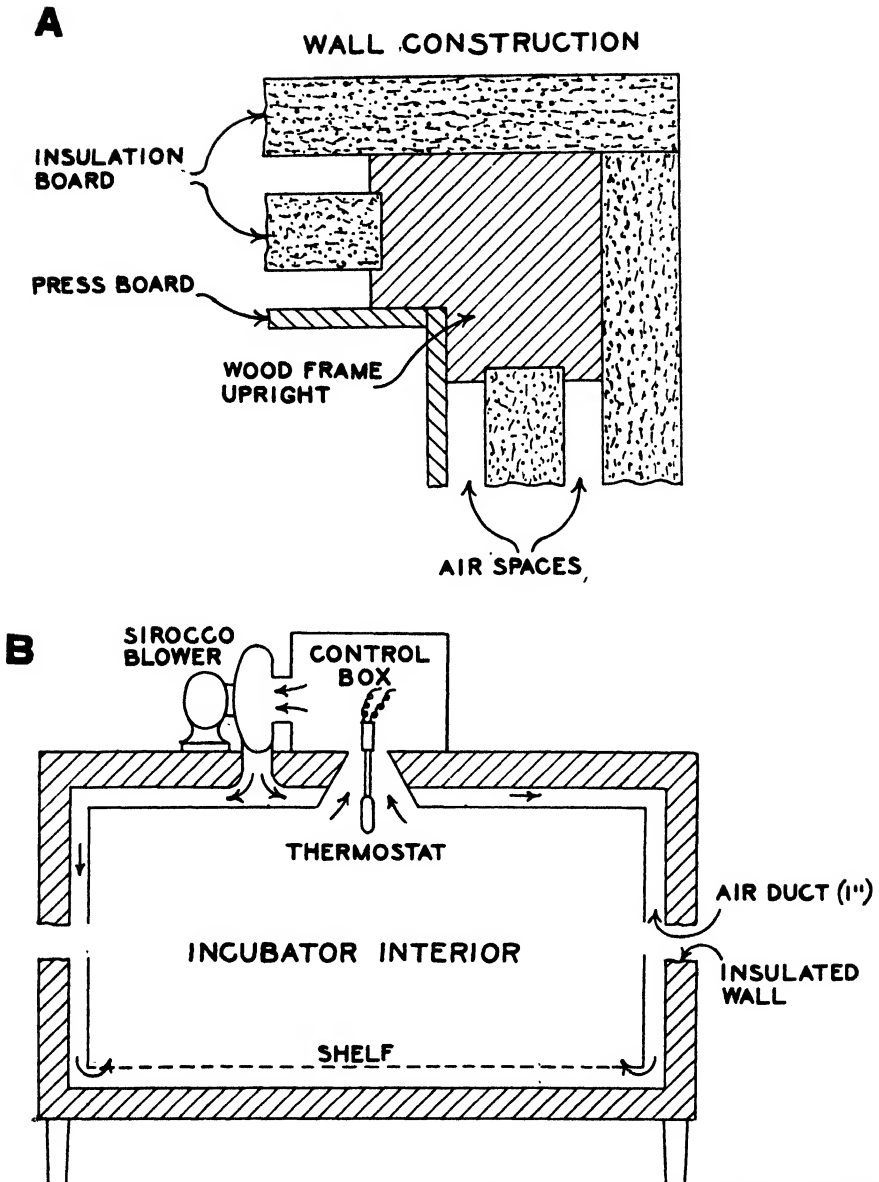


FIG. 1. A. Diagram showing construction of incubator walls. B. Diagram showing interior of incubator.

Temperature control in the compartment, the incubator being in a room at ordinary temperature, and a glass-mercury thermostat sensitive to  $0.1^{\circ}$  C. being used, is to  $\pm 0.15^{\circ}$  C. at a temperature of  $35^{\circ}$  C. Enough heat is furnished by the 150-watt heater (and by the blower) to maintain this tem-

perature. No difference in temperature could be detected with a thermometer in different parts of the incubator. Air circulation, or draft in the compartment, on the other hand, is barely perceptible to the hand, and, being directed from below upward, it cannot lift even fine powders from their containers.

Illustrative of the precision obtainable through use of this incubator the following may be cited. The dry-weights in milligrams of two pairs of duplicate cultures of *Aspergillus niger*, kept in different parts of the incubator, were  $609.1 \pm 3.5$  per cent for the one pair, and  $630.8 \pm 5.3$  per cent for the other pair. The average for the two pairs was  $645.0 \pm 2.2$  per cent. In another case the averages of the two pairs were  $476.7 \pm 1.5$  per cent and  $467.8 \pm 0.3$  per cent., the average for the two pairs being  $472.3 \pm 1.0$  per cent.—ROBERT A. STEINBERG, Bureau of Plant Industry, Washington, D. C.

*Stereum gausapatum*, Cause of Heart Rot of Oaks.—Numerous fungi cause decay in living hardwood trees of the eastern United States and usually several species are responsible for similar types of decay. For example, *Polyporus sulphureus*, *P. spraguei*, and *Daedalea quercina* all produce brown carbonizing rots, and *Fomes everhartii*, *F. igniarius*, and *F. rimosus* cause soft, stringy decays. The “piped” or white-lined type of decay also is produced by several different fungi so that it is difficult to determine the causal organism from decayed wood alone. On account of this complication the Division of Forest Pathology is attempting to identify culturally the causal organism of each decay collected, by comparison with a reference collection of cultures made from sporophores of fungi known to induce such decay.

In the spring of 1933, a large percentage of the cultures obtained from decays of oak was of a fungus that differed from any of the well-known decay fungi. In initial stages of decay this fungus forms white lines through the sound wood causing a mottled appearance. These white lines or channels usually follow the spring wood vertically, but they branch frequently and sometimes penetrate through the annual growth rings. Later, much of the spring wood becomes decayed and, in the final stages, all the wood becomes light colored and brittle.

A *Stereum* fruiting body, later determined as *S. gausapatum* Fr. by L. O. Overholts, was associated on one tree with the decay that gave the above-mentioned fungus. The fungus isolated from the decay was identical with spore cultures of *S. gausapatum* made by Lawrence L. Sluzalis in 1932. The writer has since obtained 5 additional spore cultures of *S. gausapatum* and all of these seem to be the same as the decay fungus. Several of the cultures isolated from decayed wood have formed abnormal *Stereum*-like fruiting bodies in culture, but no spores were produced on them.

On examining a large collection of unidentified decay cultures that have accumulated since 1928, it was found that many were of this same fungus. These, together with those obtained by the writer, indicate that *Stereum gausapatum* is common as a heart-rotting organism in living oak trees. It has been obtained in culture from 6 *Quercus prinus*, 13 *Q. alba*, 5 *Q. coccinea*, 7 *Q. velutina*, 4 *Q. rubra*, and 4 *Quercus* sp. These were collected in North Carolina, West Virginia, Virginia, Pennsylvania, New Jersey, New York, and Connecticut.<sup>1</sup>

Nelson and Hedgecock examined a third-generation coppice growth at Mt. Alto, Pennsylvania, in 1928, and obtained cultures from decay in 17 of the trees. Sixteen of these cultures were *Stereum gausapatum*, which indicates that the fungus may be of increasing importance in sprout-grown oaks. Decay in these trees is thought to have gained entrance from the old stumps, and in all of the other cases, with the exception of 4 fire-scarred trees, the entrance of decay seemed to be from old stumps or through dead companion sprouts.

The height of the decay in the trees at Mt. Alto was not recorded, but observations by the writer in 1933 indicate that it advances well above stump height. In 4 cases the decay was taken from butt logs left as culls by logging crews.

Additional field studies are being conducted that should bring out more definitely the relative importance of this and other decay-producing organisms. *Stereum spadiceum* Fr., which is given as a synonym of *S. gausapatum* by Burt, is the cause of decay in standing oaks in France.<sup>2</sup>—Ross W. DAVIDSON, Division of Forest Pathology, Bureau of Plant Industry, Washington, D. C.

*Stem-spot of Rhubarb*.—A disease of *Rheum rhaponticum* has been known in California since 1914, but to the writer's knowledge, has never before been reported. The disease is now prevalent in many rhubarb fields and is most severe on the first cuttings. It is characterized by oval to elliptical reddish or reddish brown spots 1–15 × 1–3 mm. in size, or larger when they coalesce. A species of *Phyllosticta* (tentatively determined as *Phyllosticta straminella* Bres.) has been repeatedly isolated from the lesion on the petioles. It is probable that the stem-spot herein described may be identical with this disease, as reported by Stevens.<sup>1</sup> He states that *P. strami-*

<sup>1</sup> The writer is indebted to A. D. Chapman, George G. Hedgecock, R. M. Nelson, L. W. R. Jackson, and P. Spaulding for collecting many of the specimens and to Chapman, Jackson, and Nelson for culturing some of them.

<sup>2</sup> Les pourritures du bois de chêne sur pied. Bull. 13, Comm. d'études des ennemis des arbres, des bois abattus et des bois mis en oeuvre. Ann. de l'École Nat. des Eaux et Forêts et de la Stat. de Recherches et Exp. Forest., iv, 2, pp. 365–380, 400–401, 406–407. 1932.

<sup>1</sup> Stevens, F. L. Two Illinois rhubarb diseases. Illinois Agr. Expt. Sta. Bul. 213: 299–312. 1919.



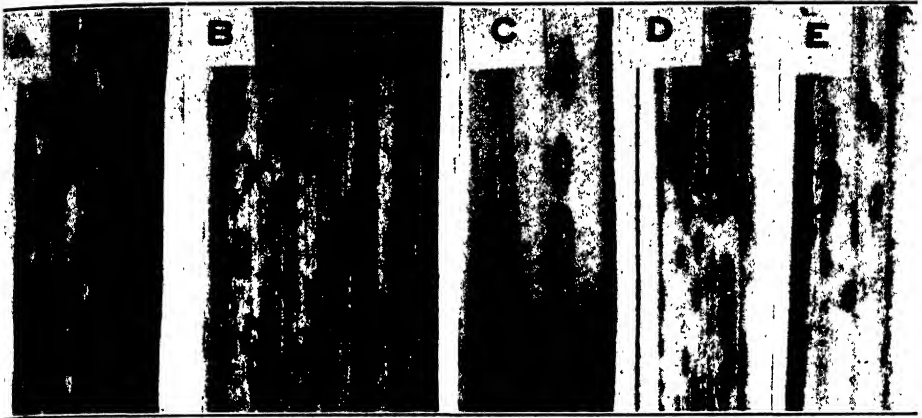


FIG. 1. A-B. Natural lesions on petioles of rhubarb. C. Inoculation through a wound using spores of *Phyllosticta straminella*. Photographed after 30 days. D-E. Stems inoculated with spores of *P. straminella* on cotton saturated with inoculum and wrapped about the petioles with paraffin paper. Photographed after 52 days.

*nella*, while mainly affecting the leaf blade, has been found also upon the petioles. He gives no illustration or description of these stem lesions. In California the stem-spots are of more economic importance, although the leaf-blade and veins are attacked and furnish abundant sources of inoculum for infecting the petioles. In 60 cultures made from the stem lesions, 35 gave *Phyllosticta straminella*. The other cultures were miscellaneous fungi, as *Alternaria*, *Rhizopus*, *Cladosporium*, and *Penicillium*. Artificial inoculations and controls on the petioles were made through wounds, by atomizing, and by placing cotton saturated with inoculum in contact with the uninjured petioles wrapped with paraffin paper. More or less typical lesions developed in 30 to 45 days. Through the courtesy of J. C. Gilman a herbarium specimen of *Phyllosticta straminella*, collected August 9, 1927, was received by the author November 22, 1932. This specimen was number 1624, United States Department of Agriculture, and Iowa State College Mycological and Plant Disease Survey of Iowa.<sup>2</sup> Eleven different dilution plates were made from this 5-year-old herbarium material and 524 colonies of *P. straminella* grew. Transfers from selected colonies were pathogenic on rhubarb. This paper is preliminary to a more detailed study.—CLAYTON O. SMITH, University of California, Citrus Experiment Station, Riverside, California.

*Observations on Gymnosporangium germinale*.—A dissemination distance for *Gymnosporangium germinale* (Schw.) Kern, the causal organism of quince rust, from *Juniperus communis* var. *depressa* Pursh to apples of certain varieties has been determined at Orono, Maine. The orchard under

<sup>2</sup> Gilman, J. C., and W. A. Archer. The fungi of Iowa parasitic on plants. Iowa State Col. Jour. Sci. 3: 299-507. 1929 (See page 376.)

consideration is slightly west of north from a stand of heavily infected *Juniperus*. For 3 consecutive seasons fruit infected by the quince rust organism has been found throughout the Red Delicious part of the orchard. In a survey of the surrounding land, no other stand of *Juniperus* has been found. The approximate shortest distance between the infected *Juniperus* and infected apple trees is 3,900 feet. The extreme distance between the infected hosts is over 4,500 feet. The degree of fruit infection per tree varied from none to approximately 5 per cent by fruit count on the variety, Red Delicious, in 1933. Only an occasional tree with a poor fruit set escaped infection. The local weather data during the dissemination period are given below:

TABLE 1.—*The temperature extremes, precipitation, and direction of the wind for 8 consecutive days beginning May 24, 1933*

Date	Temperature degrees F.		Precipitation	Prevailing wind
	Maximum	Minimum		
			<i>Inches</i>	
May 24 .....	74	48	0.14	S.
" 25 .....	76	45	0.00	S.
" 26 .....	86	48	0.00	S.E.
" 27 .....	52	38	0.88	S.E.
" 28 .....	51	38	0.00	N.
" 29 .....	74	36	0.00	S.E.
" 30 .....	55	46	0.00	S.E.
" 31 .....	60	47	0.91	S.E.

The weather data coincide with those considered to be favorable for sporidia dissemination, germination, and infection of the host during at least part of the interval of maximum sporidia production and fruit susceptibility. The direction of the wind was relatively favorable for dissemination of sporidia from the infected *Juniperus* to the orchard under observation. During the past 3 years fruit of the following varieties of apples infected by *Gymnosporangium germinale* have been observed in Maine orchards: Baldwin, Bellflower, Cortland, Duchess, Early Red McIntosh, Golden Delicious, Gravenstein, Jonathan, McIntosh, Red Delicious, Starking, Tolman Sweet, Twenty Ounce, Wagener, Wealthy, and Winter Banana. In Maine the most susceptible commercial varieties apparently are Red Delicious, Golden Delicious, Cortland, and Wealthy. The region of infection is at or near the calyx end of the apple fruit. Lateral infections, such as are shown by Thomas and Mills,<sup>1</sup> have not been observed. Aeciospore production on the apple fruit is not common.—F. H. STEINMETZ and M. T. HILBORN, College of Agriculture, University of Maine, Orono, Maine.

<sup>1</sup> Thomas, H. E., and W. D. Mills. Three rust diseases of the apple. New York (Cornell) Agr. Expt. Sta. Mem. 123. Plate II. 1929.

**REPORT OF THE ANNUAL MEETING OF THE SOUTHERN  
DIVISION OF THE AMERICAN PHYTOPATHO-  
LOGICAL SOCIETY**

The annual meeting of the Southern Division of the American Phytopathological Society was held in conjunction with that of the Association of Southern Agricultural Workers in the Peabody Hotel, Memphis, Tennessee, January 31 to February 2, 1934.

Three half-day sessions of the Society were scheduled but, on meeting the first day, it was found more convenient to combine the three scheduled programs into two and to terminate the meetings in so far as our Division was concerned on the afternoon of February 1.

At the business meeting, on the afternoon of January 31, the following officers were elected to serve the Society for the next year:

*Chairman* ... D. C. NEAL, U. S. Cotton Breeding Station,  
Greenville, Texas.

*Secretary-Treasurer* . . . E. C. TIMS, Louisiana Agricultural Experi-  
ment Station, Baton Rouge, Louisiana.

*Councilor* . . . V. H. YOUNG, Arkansas Agricultural Experi-  
ment Station, Fayetteville, Arkansas.

This was the first meeting of the Southern Division to be held in conjunction with the meetings of the Association of Southern Agricultural Workers since such a practice was abandoned several years ago in favor of meetings to be held during the summer months. The summer meetings, however, were never very successful because of the difficulty experienced in securing an adequate attendance, most of the workers finding it difficult to leave their research projects during the summer months at the time when they were most active and required the closest supervision.

The attendance at this meeting was small, as was naturally to be expected after such an interval, and also because of the world-wide depression and its effect on the budgets for traveling purposes, which have been materially reduced if not entirely eliminated in all Southern institutions. In spite of the small number present, a total of 21 excellent papers were presented in a manner characterized by a delightful informality that contributed greatly to sustaining interest throughout the sessions. Ample opportunity was afforded for discussion, which was indulged in freely by everybody. Abstracts of the papers follow the present report.

L. E. MILES, *Secretary*

## ABSTRACTS

*Studies on Potato-Scab Control.*—J. J. TAUBENHAUS. In previous experiments by the writer no appreciable control of potato scab (*Actinomyces scabies*) was obtained when sulphur was used at the rates of 1,000 to 5,000 pounds per acre and applied broadcast to soils rich in calcium and of an alkaline reaction. During 1933, a series of potato scab experiments with sulphur was carried out in different sections of East and East Central Texas and under a variety of soil conditions ranging from neutral to alkaline in nature. The sulphur in these experiments was applied in the furrows some two or three weeks before planting at rates varying from 500 pounds in the neutral soils to 1,500 pounds per acre in the calcareous soils. The potatoes were then cut and planted in the usual way and the crop was given ordinary field culture.

Definite control of potato scab was obtained in all the tests and under all the soil types where the sulphur was applied in the furrow. This control of potato scab in these experiments was independent of soil reaction, particularly in the calcareous soils where no change of pH was obtained when the sulphur was placed in the furrows. In this connection, it should be noted that slight reduction in yield of tubers was obtained in the sulphur-treated plats, although this was to a certain extent compensated by smoother and better quality No. 1 tubers where the sulphur was used.

*Studies on Nailhead Spot of Tomatoes.*—GEORGE F. WEBER. Nailhead spot is a common disease of tomatoes found in the Southeastern and Gulf States, causing considerable damage annually. Confusion manifested in the literature concerning the causal organism and certain other closely related organisms on tomatoes has prompted this study and the following conclusions: The fungus causing nailhead spot, known in literature as *Macrosporium tomato* Cke., has been shown through cultural studies to be a species of *Alternaria*, and the new combination, *Alternaria tomato* (Cke.) is proposed. It is morphologically and physiologically distinct from the early blight parasite, *Alternaria solani* (E. & M.) J. & G., with which it is often confused. Both parasites are found on individual tomato plants, and causes distinguishable, but similar, diseases on stems, foliage, and fruit. These parasites show notable differences and variability in pure culture. Spores collected in nature and those developed in pure culture are consistently comparable for each parasite, but distinct from each other. None of a number of other species of *Alternaria* isolated from tomatoes have proved to be parasitic through artificial inoculation.

*Studies on the Control of the Seedling Blight of Rice in Arkansas.*—E. M. CRALLEY. The seedling blights of rice are widely distributed throughout the rice-growing areas in Arkansas. Most of the blighting is due to *Helmintosporium oryzae* von Breda de Haan. In 1933 *Fusarium* and a nonfruiting, sclerotium-bearing fungus caused considerable blighting. Thus far the seedling blights have been controlled most effectively by (1) the use of good seed, and (2) by delaying the planting date until the soil temperature is favorable for the vigorous growth of the young rice seedlings. According to preliminary greenhouse and field experiments the most serious blighting takes place when the soil temperature is between 16° to 23° C. The seedling diseases were not satisfactorily controlled by treating rice seed with formaldehyde, Ceresan 1, Ceresan 2, or copper-lime dusts. No seedling blighting was observed in 1933 in experimental plots where the rice was sown early in the season directly in the water.

*Treatment of Sweet-Potato Plants for the Control of Black Rot.*—L. E. MILES. Spread of black rot on sweet-potato plants during transit is a cause of great loss to the grower of sweet-potato plants for the market, regardless of however careful and con-

scientious he may be in applying the control measures already in practice against the disease in the seed bed, field, and storage in that a single infected plant may often spread the infection throughout an entire shipment while the plants are in transit from grower to purchaser. Terminal inspections now in vogue in many States cause the condemnation and loss of such shipments; or, if they are not intercepted, the loss is suffered by the purchaser.

This loss, as shown by tests conducted at this station in 1932 and 1933, may be prevented by the grower by dipping the plants, prior to packing for shipment, into a 20-20-50 Bordeaux-mixture solution, the stems and roots alone being immersed, or by immersing the entire plants in a 25 per cent copper-lime dust. Infection of the plants by spores of the disease organism present on their surfaces is prevented. Such treatment, moreover, does not injure the plants materially, even when the treated plants are in shipment in a closed container as long as 5 days, as is shown by stand and yield records taken on treated and nontreated plants. Such treatment is recommended to the grower of plants for either sale purposes or for home use in order to prevent dissemination of the disease organism to clean plants through any chance infection that may be present in the seed bed. There is evidence that this treatment will delay infection from infested soil to an extent that may make its use practicable when clean plants are put out on land known or suspected to harbor the disease, though this phase of the work has not yet been sufficiently worked out.

*Preliminary Report on an Anthracnose of Lima Bean.*—T. D. PERSONS. On August 20, 1932, specimens of diseased Lima beans were received from Lyman, Mississippi. The diseased areas were reddish brown and of irregular outline. Their size varied from very small reddish specks to areas as wide as the width of the pod, and in some cases these areas had coalesced to such an extent that almost the entire pod was affected. There was an abundance of pink spore masses in the diseased areas, and examination with a hand lens revealed the presence of dark setae around the edges of the spore masses.

The fungus causing this disease has, apparently, not been definitely determined; it would seem, however, to be a *Colletotrichum*. In 1883, M. C. Cooke collected a fungus on Lima beans in South Carolina and described it as *Vermicularia polytricha*. His description in Grevillea, vol. 12, p. 24, though very meagre, fits this fungus very well, especially as to spore size and shape. His spore measurements are given as  $30 \times 4 \mu$ . In 1911, Heald and Wolf described a fungus causing a stem canker of Kentucky Wonder beans in Texas as *Colletotrichum caulicolum*. Their description in Mycologia, vol. 3, p. 10, also fits this fungus very well. Their spore measurements are given as  $18-30 \times 3.5-4 \mu$ . The size of the spores of the fungus causing the disease in Mississippi, according to measurements recently made, varies from  $21.6$  to  $28.8 \mu$  in length and the width in all cases was approximately  $3.6 \mu$ . The spores in nearly all cases were more or less sickle-shape. In the Plant Disease Reporter, vol. XV, p. 113, W. D. Moore reports a pod blight of Lima beans in North Carolina and Virginia. His description of the symptoms on the pods agrees very closely with those of this disease. This report also calls attention to the resemblance between the causal fungus and the descriptions of *Vermicularia polytricha* and *Colletotrichum caulicolum*.

The writer plans to continue his studies of this disease in an effort to definitely identify the causal organism and to formulate control measures.

*A Report on the Use of Creosote Oil to Control San Jose Scale and Peach Leaf Curl.*—W. W. STANLEY, S. MARCOVITCH, and J. O. ANDES. Tests during the last two years have shown that creosote oil is toxic to San Jose scale and to peach-leaf curl. The

control obtained was in direct proportion to the amount of creosote oil in the spray. The strongest spray used was 8 per cent creosote oil, which gave 91.09 per cent control of San Jose scale and 96.54 per cent control of peach-leaf curl. Combinations of small amounts of creosote oil and oil emulsion were much more toxic than when the materials were used separately or at higher concentrations. The combination of 1 per cent creosote oil and 3 per cent oil emulsion gave 99.08 per cent control of San Jose scale and 97.96 per cent control of peach-leaf curl. Creosote oil and coal-tar distillate (Arbo) appeared to be equally toxic.

The creosote oil used in the tests is a product of destructive distillation of hardwoods and was obtained from the Tennessee Eastman Corporation, Kingsport, Tenn.

*Recent Investigations of Cotton Root Rot in Texas.*—D. C. NEAL. It has been determined that the fungus causing root rot overwinters in the soil chiefly in the sclerotial or strand-sclerotial stage, and as dormant mycelium in plant roots infected from the previous year's crop. Numerous tests have shown that the sclerotia and the strands are capable of persisting in the soil in a viable condition in the absence of live host material for long intervals, doubtless for several years, and that these stages of the pathogen occur in some areas at considerable depth in the soil.

Careful records of infection show that root-rot spots, though usually persisting in alfalfa or cotton fields for several years and enlarging each year by a new belt of growth, may sometimes disappear for a season or longer and then reappear as small centers of infection that begin to expand with renewed vigor. Recent studies of the growth of the fungus on culture media have shown that the above spot behavior may be associated with a more or less normal growth cycle of the fungus itself. For example, fresh isolations of the fungus usually make rapid growth and produce strands and sclerotia in abundance, whereas cultures maintained for several months frequently become attenuated or dissociated and lose their capacity to produce strands or sclerotia and yield only fine white mycelium. Since it is definitely established, even in the case of isolations obtained from widely separated localities, that the fungus may become attenuated *in vitro*, it is thought that at some period of its growth it may assume a similar morphology in nature, a stage that undoubtedly would be unfavorable for prolonged viability in the soil, thereby resulting in a breaking up or disappearance of the infection.

Additional experiments conducted during the seasons of 1932 and 1933 with ammonia-nitrogen carriers have further confirmed the toxic effect of ammonia on the fungus. Also, in studies of the toxic effect of ammonia water on sclerotia, strands, and mycelium of the fungus in cotton-root sections, it was found that the sclerotia and the strands are killed after short exposure to 1 and 2 per cent solutions, but that a longer time and a higher concentration is required to kill the fungus completely in infected root sections. In field experiments groups of root-rot sclerotia buried in Wilson clay soil at a depth of 12 inches were killed with 3 per cent ammonia water after an exposure of 5 days, and in other groups similarly treated, but buried at a depth of 18 inches, the number remaining viable was perceptibly reduced.

Further observations on the effect of field applications of ammonium sulphate, ammonium hydroxide, anhydrous ammonia, and other ammonium compounds continue to show that ammonia is effective in reducing the disease in certain sections of the infested districts of Texas. In heavily infested plots treated in 1932 and 1933 with ammonia and ammonium compounds, significant reduction of the disease was obtained, and the residual effect of the 1932 application was still marked during 1933. The most favorable results, however, both from the standpoint of increased yields of seed cotton and in a reduction of root rot have been obtained where ammoniacal materials were used in Wilson clay or related types of soil.

*Reversible Vegetative Dissociation of Strains of Phymatotrichum omnivorum.*—WALTER N. EZEKIEL and J. J. TAUBENHAUS. Strains of *Phymatotrichum omnivorum*, when transferred by portions of the hyphal mats in agar slants, have retained their original cultural characteristics for more than 5 years. It has been possible, however, regularly to select substrains of dissimilar characteristics, by growing the fungus in flask cultures and making repeated transfers, in one series using as the inoculum sclerotia from the first flasks to produce these bodies and in a parallel series using as inoculum portions of the hyphal mat from the last flask to produce sclerotia. Strains retained their characteristics when transferred from tube to tube; but further selection could still be made from them of new substrains that reverted to the original characteristics. These reversible dissociations from the strains may be considered as possible because of nuclear heterogeneity of the original mycelium, arising from previous anastomoses between various strains, or from occasional mutations.

*Insects as Possible Distributing Agents of Cotton Root Rot Caused by Phymatotrichum omnivorum.*—J. J. TAUBENHAUS and L. D. CHRISTENSON. A number of beetles, *Blaptinus fuscus*, and a species of *Harpalus*, which normally feed on cotton plants, were placed in screened cages in the laboratory and made to feed on cotton roots freshly infected by the root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar. These insects fed copiously on the infected cotton roots and on the *Phymatotrichum* strands. Likewise, a number of cotton-leaf-eating larvae were fed on cotton leaves that had been painted over with a heavy suspension of the spores of *P. omnivorum*. The fecal pellets or the entire insects were then surface sterilized in a bichloride solution and cultured on nutrient agar in Petri dishes. At no time was *P. omnivorum* recovered either from the fecal pellets or from the insects thus treated. From this it appears that cotton-feeding insects do not spread *Phymatotrichum* root rot of cotton.

*Further Studies on the Toxic Principles that Determine Immunity of Monocotyledonous Plants from Phymatotrichum Root Rot.* WALTER N. EZEKIEL, J. J. TAUBENHAUS, and J. F. FUDGE. It has been shown that juices from the roots of monocotyledonous plants contain materials that inhibit growth of the root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar. Continued studies, directed toward isolation and identification of these toxic elements, have been carried out with fractions of juices, concentrated under reduced pressure and added to nutrient solutions. Ether extracts from the juices of 4 monocotyledonous plants (onions, corn, canna, and *Hemerocallis*) were generally toxic to the fungus. The aqueous residues invariably were toxic. Ether fractions from 4 dicotyledonous plants (carrots, turnips, potatoes, and sweet potatoes) were not toxic, except for potato juice at higher concentrations in one experiment, while the aqueous residues were toxic. These results suggest that the materials that make monocotyledonous plants immune from *Phymatotrichum* root rot are those present in the ether-soluble fractions of juices from the roots of these plants. The toxic elements in the autoclaved, concentrated, aqueous fractions are apparently present in comparable amounts in fractions from both susceptible and immune plants and presumably are not involved in the immunity of monocotyledonous plants.

*Insects as Possible Distributing Agents of Cotton Wilt Caused by Fusarium vasinfectum.*—J. J. TAUBENHAUS and L. D. CHRISTENSON. A number of cotton-feeding insects were brought into the laboratory and placed in screened cages containing roots, stems, or leaves from cotton plants definitely known to be affected by *Fusarium* wilt (*Fusarium vasinfectum* Atkinson). The fecal pellets from the insects were then surface-sterilized and cultured on nutrient agar in Petri dishes. Some entire insects, when gorged with

the infected cotton material, were cultured also in the same manner as the fecal pellets. Good growth of *Fusarium vasinfectum* was recovered from the entire insects or the fecal pellets of the following: white grubs (*Phyllophaga crassissima*), grasshoppers, stem borers (*Ataxia crypta*), boll weevil larvae (*Anthonomus grandis*), and boll worms (*Heliothis obsoleta*). No *Fusarium* growth was obtained from wire worms.

In other experiments cotton insects were fed on infected cotton material and, after 24 hours' feeding, were killed in a bichloride solution. The alimentary canal, aseptically dissected out, was then cut up in small portions and cultured. *F. vasinfectum* was recovered from all parts of the gorged alimentary canals of the larvae of *Schistocera americana* and *Alabama argillacea*. In similar experiments insects were fed on infected cotton material, then starved for several days and all the fecal material was defecated. These insects were then killed in bichloride solution, the alimentary canal aseptically dissected out, cut up in small portions, and cultured. No *Fusarium* growth was obtained from the sections of the alimentary canal void of infected cotton-plant food. This indicates that the fungus, *F. vasinfectum*, the cause of cotton wilt, is able to pass through, in a viable condition, the alimentary canal of certain cotton feeding insects. The fungus thus recovered was capable of infecting normal cotton plants. These experiments show that many cotton-feeding insects may act as disseminating agents of cotton wilt.

*Studies on the Fusarium Wilt of Cotton.*—V. H. YOUNG and J. O. WARE. Cotton wilt and "rust" or potash hunger are commonly associated on much of the sandy alluvial soil of Arkansas. Often root knot is a factor. Since cotton wilt becomes unusually severe when either root knot or rust is present, their control is prerequisite to cotton-wilt control. No better method of control for root knot has been found than the use of resistant or immune crops and weed control in rotation with cotton. Work at the Cotton Branch Experiment Station in Lee County in Eastern Arkansas and elsewhere in Central and Eastern Arkansas had demonstrated the efficiency of liberal applications of potash either in mixed fertilizer or alone in the form of muriate of potash or kainit for the control of cotton wilt and rust. Stable manure at the rate of 10 tons per acre gave excellent control of rust, but data concerning its effect on the incidence of cotton wilt were not clear cut. Cotton seed meal at the rate of 600 pounds per acre was entirely ineffective so far as either wilt or rust was concerned. Nitrates or phosphates either alone or used together not only gave no control of either potash hunger (rust) or wilt but actually appeared to unbalance conditions so that more rust and wilt resulted than was present on the unfertilized checks.

In 1933 cotton wilt was more severe than for several years. Unfertilized checks of susceptible varieties of cotton, such as Trice 304 and Missdel 2, showed about 36 per cent of plants affected with wilt. Use of 600 pounds per acre of a 6-8-12 (NPK) fertilizer gave complete control of potash hunger and reduced wilt to about 8 per cent when Trice 304 cotton was used, to about 16 per cent when Missdel 2 was used, and to about 2 per cent when Arkansas Rowden 2080, a wilt-resistant strain, was employed. Kainit or muriate of potash in amounts sufficient to supply potash in amounts mentioned above gave similar control of potash hunger and cotton wilt but gave lower yields than did a complete fertilizer. Results of 5 years' work on the control of wilt and rust indicate that commercial-cotton wilt and rust control on the sandy alluvial soil of Arkansas is best secured by the combination of a suitable wilt-resistant variety of cotton with the judicious use of a suitable fertilizer containing enough potash to supply the potash needs of the soil.

*Seed-Treatment Studies with Fungicidal Dusts at the Arkansas Experiment Station.*—V. H. YOUNG.\* Seed-treatment studies with oats and corn were carried out at the



Main Experiment Station in northwest Arkansas and seed treatments with cotton at the Main Station and at the Cotton Branch Station in eastern Arkansas.

Organic-mercury and formaldehyde dusts gave almost perfect control of oat smut (*Ustilago avenae* and *U. levis*) with corresponding yield increase. There also was some indication of other benefits than smut control. Slight stand increases resulted from treatment of apparently healthy seed of Neal's Paymaster Corn with various mercury-containing dusts when seed was planted early in the season; but later plantings were not benefitted and in no cases were yield increases closely correlated with increases in stand. It was concluded that corn-seed treatment, under the conditions of the experiment, were of little or no value. The oat- and corn-seed-treatment studies were the joint work of C. K. McClelland and the writer.

Earlier cotton-seed treatments made by the sulphuric acid-delinting method, combined in some cases with the use of mercuric chloride, hastened germination and gave excellent control of seed-borne diseases, but growers have been unwilling to adopt them. Later experiments with Germisan and Uspulun in solution gave good control of angular leaf spot of cotton, increased the stands, and appeared to benefit yields often as much as 20 per cent. Liquid treatments for cotton have never appeared acceptable to Arkansas growers and the work was discontinued.

During the past 4 years, organic-mercury dusts, formaldehyde dust, red oxide of copper, and monohydrated copper sulphate have been used, the two latter dusts only in 1933. Dusts containing ethyl mercury chloride and ethyl mercury phosphate have given consistent stand increases, especially with early planted cotton, often amounting to as much as 50 to 100 per cent, and have given considerable control of angular leaf spot of cotton. Other dusts employed were either detrimental to germination of cotton or had no discernible effect. In spite of stand increases from cotton-seed treatments, very few actual yield increases have been recorded. It is believed that failures to secure definite yield increases in correlation with stand increases is due to the unusual ability of the cotton plant to adjust itself to available space unless excessively long skips exist.

*Observations on the Control of Black Rot of Grapes.*—V. H. YOUNG. In the grape-growing section of northwest Arkansas, black rot, caused by *Guignardia biduellii*, is the most serious disease of Concord grapes. Although a schedule of sprays consisting of 4-4-50 Bordeaux mixture just before blooming, just after blooming, and about 10 days or 2 weeks later has ordinarily controlled the disease, many growers formerly failed to secure control due in some cases to unusually unfavorable location of their vineyards and in others to poor spraying or improper timing. Belief that one or more additional preblossom sprays were necessary for black-rot control apparently arose from failure of these growers to control the disease and from the recommendations of certain eastern stations where an additional preblossom spray is often recommended. Spraying experiments, extending over the period 1929-1933 using from 1 to 3 preblossom sprays and heavier dosages of copper and lime than those found in a 4-4-50 Bordeaux mixture, gave no better control than was secured with the standard schedule recommended for Arkansas growers. Likewise, during the 5 years of the experiment a late, nonstaining spray consisting of 1 pound of neutral acetate of copper to 50 gallons of water did not improve control, since, in every case, black-rot spread was checked by the dry weather of midsummer, making late spraying for the disease unnecessary.

In 1930 severe freezing in midwinter resulted in much killing back of grape vines and a subsequent unusual development of suckers that arose at or often below the soil line. On May 23, 1930, large numbers of black-rot cankers were found at the base of these suckers. They were located in an area extending from a few inches above the soil

line to just below it and were covered with pycnidia containing mature spores. At this time black-rot lesions on the upper parts of the vines were just appearing and showed no fruiting bodies. It appears that infections on suckers must have resulted from spores washed onto the young sprouts just as they pushed from the soil. Since large numbers of black-rot mummies from the previous season were present on the ground, it is suggested that these unusually early infections probably arose from conidia or ascospores borne on mummies of the previous year. Rapid growth of a corky layer on the suckers soon isolated the diseased tissues and by midseason they had almost disappeared.

*Control of Fire Blight.*—H. R. ROSEN. Investigations on the possible prevention of fire blight by the application of sprays applied at times when bacterial dissemination occurs have been continued. As in 1932, excellent control was obtained in 1933 in a Jonathan orchard by the application of 1-3-50 Bordeaux mixture applied as follows: First, as a cluster-bud spray; secondly, when about 20 per cent of the blossoms were fully opened; thirdly, when about three fourths of the blossoms were fully open; fourthly, as a calyx spray; and fifthly, as a first-cover spray.

A brief summary of the results shows that on the 56 check trees that received lime-sulphur spray applied as a cluster-bud, calyx, and first cover spray, the average amount of blighted clusters per tree was 30 per cent, varying from a trace to destruction of almost all blossoms. On the 87 trees sprayed with Bordeaux mixture the amount of blossom blight was restricted to an insignificant fraction of 1 per cent.

Twig blight was negligible on the Bordeaux-sprayed trees, while on the check trees it was largely confined to sprouts that arose from blighted clusters.

Russeting of the fruit on the Bordeaux-sprayed trees was more abundant in 1933 than in 1932, although the amount of injury was not sufficient to reduce seriously the marketable value of the fruit.

*High Points in Apple Spraying in Tennessee.*—J. O. ANDES. Blossom blight was partially controlled by spraying with 1-3-50 Bordeaux mixture when the blossoms were two thirds open. Canker treatment as a supplementary measure, using a 53 per cent zinc chloride solution, is recommended. On Golden Delicious, flotation sulphur, 8 pounds to 50 gallons, was found an effective substitute for liquid lime-sulphur and Bordeaux mixture in scab control. Bordeaux mixture applied to fully grown fruit of this variety did not cause russeting. Up to 9 sprays was required to give adequate control of all diseases and insects on all varieties under all conditions. Some of the sprays can be omitted under certain conditions without a decrease in the effectiveness of control.

*Control Measures for Rosette of Blackberries and Dewberries in Louisiana.*—A. G. PLAKIDAS. Studies on rosette in Louisiana have shown that: (1) the cause of the disease is a species of *Cercospora*; (2) infection takes place in the spring on the primocanes, the infected canes remaining normal in appearance until the following spring when, on resuming growth, they develop the rosette symptoms; (3) the period of infection is limited from about the middle of March to the first week in June; (4) the disease can be effectively controlled by spraying the primocanes with Bordeaux during the period of infection. From these findings, a system of control has been devised that, while still in the experimental stage, has proved very effective. This system is a combination of pruning and spraying. All the primocanes are pruned to the ground about the middle of May. Then, the new canes that develop from the pruning date to about the middle of June are sprayed with Bordeaux. Usually, two sprayings seem to suffice. Because of the long growing season in Louisiana (the plants continue to grow as late as Novem-

ber) the removal of the early primocanes apparently does not weaken the vigor of the plant.

*Stilbum on Fig in Louisiana*.—EUGENE C. TIMS. Cross inoculations made on other hosts with *Stilbum cinnabarinum* (Mont.) from fig indicate that the cultures used are capable of attacking pear (Pineapple variety) and to a limited extent rose (Killarney variety), but are not parasitic on plum, peach, or pecan. The Celeste fig is quite susceptible, but some of the larger, coarser varieties, such as the Brunswick, are more resistant. Infection of the fig may take place through wounds or leaf scars, as has been demonstrated by various types of inoculations. Pruning out infected branches and painting the cut ends with outside paint has not been effective in eliminating the fungus from one small group of fig trees. Other control measures are being tried.

*New or Unusual Diseases Reported or Observed in Mississippi in Recent Years*.—T. D. PERSONS. The following is a list of new or unusual diseases reported or observed in Mississippi in recent years by various workers. In some cases the diseases are new for the hosts, while in others they are known to occur in other parts of the country but have not previously been reported from this State.

Root knot, caused by the nematode, *Heterodera marioni*, has been collected on *Abelia* sp. and on *Ginkgo biloba*. This constitutes the first report of the occurrence of this trouble on either of these hosts. The occurrence on *Ginkgo* is especially interesting because of the unique botanical position of this plant. In so far as the writer has been able to determine, this is the first report of root knot on any of the gymnosperms in the United States. Southern blight (*Sclerotium rolfsii*) is reported for the first time on China aster. Bacterial spot (*Bacterium holci*) on corn. This is apparently the first report of this trouble outside of Iowa, whence the disease was described originally. Verticillium wilt (*Verticillium dahliae*) on cotton is now known to occur in 7 counties in the State. Crown gall (*Pseudomonas tumefaciens*) on *Juniperus sabina*. First report of the disease on this host. The only other conifer known to be attacked by this disease is *Cupressus arizonica*, reported from Arizona by Brown and Evans. Orange rust (*Gymnoconia interstitialis*) is reported for the first time on loganberry. *Cercospora cruenta* is reported for the first time on mung bean. The Ascochyta blight, caused by *Ascochyta pinodella*, is reported for the first time on English peas in Mississippi. A leaf spot (*Sphacropsis salviae*) on *Salvia* sp. reported for the first time in the United States. A wilt, caused by *Fusarium oxysporum nicotianae*, on tobacco for the first time from the State. Leaf spot (*Gloeosporium aleuriticum*) on tung oil, and bacterial blight (*Bacterium juglandis*) on walnut also constitute new records for the State.

*Sorosphaera veronicae* (Schr.) on Corn or Wall Speedwell, *Veronica arvensis*.—LEROY DONALD. On May 30, 1930, specimens of *Veronica arvensis* were encountered that had conspicuous galls on their leaves and stems at or near the ground, the most common form being that of swollen, stunted stems from which sprang a few small, deformed leaves. The oldest swellings had turned brown and exhibited a snuff-like appearance. Since the galls or tumors suggested injury by nematodes, microscopic examinations were made that revealed the presence of numerous spherical and hollow spore balls within the enlarged parenchyma cells of the host. Occasionally, oblong or ellipsoidal compound structures were observed that probably resulted from a fusion of several of the spore balls. The individual spores were wedge-shape and varied from 15–20  $\mu$  in size.

The fungus was identified as *Sorosphaera veronicae* Schröter. As founded by Schröter in 1886 the genus *Sorosphaera* included only one species, namely, this one. The

genus was listed by him as one of 4 genera under the Phytomyxinae. He recognized 3 host plants in Germany on which *Sorosphaera veronicae* was parasitic, namely *Veronica hederifolia*, *V. triphylla* and *V. chamaedrys*.

Later, in 1895, Rostrup found this species on *Veronica hederifolia* in Denmark. Trotter, in 1904, reported it in *V. arvensis* from Italy. Cooke and Schwartz, while conducting an investigation of the root fungi in ponds and marshes around London, found and described from roots of a number of different grasses growing there a second species of the genus *Sorosphaera*, which was named *Sorosphaera radicalis*.

As will be noted, all the previous work on the genus *Sorosphaera*, as far as can be ascertained, has been conducted in countries other than the United States. The presence of either of the 2 species comprising the genus has never been reported from this country. *Sorosphaera veronicae* may be listed as a new fungus for the United States.

*Verticillium Wilt of Cotton in Greece*.—L. E. MILES. *Verticillium* wilt of cotton, first recorded as occurring on cotton under field conditions by Sherbakoff in Tennessee in 1928, and now known to occur on cotton in Tennessee, Arkansas, Mississippi, and California, has recently made its appearance in Greece.

In recent correspondence with the writer, Dr. J. A. Sarejanni, Plant Pathologist at the Institut Phytopathologique Benaki, Kiphissia-Athenes, Greece, advises that in September, 1932, he isolated the disease organism in 19 out of 20 cultures from Copais, Greece, from cotton grown from seed imported in the spring of 1932 from North Carolina. In later correspondence in January, 1934, he advises further that he has isolated the organism from cotton from the north in Macedonia as well as from the south in continental Greece, and always from cotton from seed imported from the United States in 1932 or 1933. *Fusarium vasinfectum*, the organism causing the very similar wilt of cotton in the United States, has been isolated only once in Greece, and in that case also by Sarejanni in 1932 from cotton grown from seed of American origin.

Sarejanni is strongly of the opinion that the disease was introduced into Greece on seed imported from America and states that "I should feel secure in saying that the introduction of American seed has brought about heavy infections in the cotton sections (of Greece)." Up to the present time, however, neither he nor the American workers who have investigated the matter have been able to establish the presence of the organism either in or on the seed nor to demonstrate whether or not the trouble is seed-borne. Moreover, the disease has not yet been reported on cotton from North Carolina nor the other Atlantic Coastal States whence the cotton seed imported into Greece presumably originated.

Cultures of the fungus isolated from cotton in Greece have been received and are identical in the growth characters of the colonies and the morphological details of the fungus with those secured from cotton in Mississippi and Tennessee.

# PHYTOPATHOLOGY

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## A MASKED STRAIN OF TOBACCO-MOSAIC VIRUS<sup>1</sup>

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### INTRODUCTION

A number of mild-mottling, attenuated strains<sup>3</sup> of tobacco-mosaic virus have been described by J. Johnson (5, 6), Johnson and Grant (7), and E. M. Johnson (4). Tobacco plants infected with these attenuated strains show less distortion and less conspicuous mottling than appear in those infected with the common, or field-type, tobacco-mosaic virus. Attenuated strains have been reported to vary among themselves (5), some consistently producing milder symptoms than others; but no strain of tobacco-mosaic virus has been described as symptomless in *Nicotiana tabacum* L.

In the course of experiments on rate of increase of tobacco-mosaic virus at high temperature, the writer isolated a strain that was able to infect, increase in, and spread systemically in *Nicotiana tabacum* var. Turkish and certain other plants, without producing appreciable mottling, blanching, distortion, or stunting. In spite of the masked character of its infection in *N. tabacum*, this strain of virus showed many of the fundamental characteristics of the common field strain, and was indistinguishable from it in some hosts. In large sets of tobacco plants inoculated with the masked strain, a few individual plants showed transient and indistinct traces of clearing of veins and of obscure mottling. It was generally not possible, however, to distinguish Turkish tobacco plants infected with this strain of tobacco-mosaic virus from noninfected, healthy plants of the same age. The presence of the masked-strain virus in infected plants was occasionally betrayed by the appearance of isolated yellow spots, which were found to be sources

<sup>1</sup> This masked strain of tobacco-mosaic virus was isolated and its properties were studied during 1930, 1931, and 1932 at the Boyce Thompson Institute for Plant Research, Yonkers, N. Y. Repurification of the original tobacco-mosaic stock virus and experiments on the origin of attenuated strains were performed in 1932 and 1933 at The Rockefeller Institute for Medical Research, Princeton, N. J.

<sup>2</sup> Published at the expense of The Rockefeller Institute for Medical Research out of the order determined by date of receipt of the manuscript.

<sup>3</sup> The results of the work reported in the present paper give evidence that these attenuated forms of tobacco-mosaic virus merit the designation "strains." For convenience, this term is used throughout.

of yellow-mosaic virus strains more or less similar to the strains arising in plants infected with ordinary tobacco-mosaic virus, as described by McKinney (9) and Jensen (3).

It is the purpose of the present paper to report experiments in which this masked strain and other attenuated strains were isolated, and to describe the properties of the masked-strain virus.

#### ORIGIN OF MASKED STRAIN

The masked strain of tobacco-mosaic virus was originally obtained in the course of the following experiment. Pieces of healthy tomato stem were inoculated with tobacco-mosaic virus of a stock isolated from a single necrotic lesion on *Nicotiana glutinosa* L. and previously used in studies of symptoms induced in a variety of host plants (2). The pieces of stem were incubated in loosely plugged, sterilized test tubes in a thermostatically controlled water-bath at 34.6° C. After 15 days' incubation, several stems were crushed and the expressed juices from each were used to inoculate plants of *N. glutinosa*. The appearance of numerous necrotic lesions on a single plant indicated that considerable virus had been produced in one of the stem pieces, but not in the others. Transfers were made from 10 of the lesions to 10 young Turkish tobacco plants. The transfer of virus from a necrotic lesion in *N. glutinosa* was made by grasping part of an infected leaf between two wooden pot labels, tearing away a bit of leaf tissue that included a necrotic lesion, crushing the tissue between the sticks, and rubbing the expressed fluid over the surface of a leaf of *N. tabacum*. This process had been found to infect at least one-half of all inoculated plants in past experiments. In this experiment, however, 9 of the 10 inoculated plants showed no symptoms as a result of the inoculation. The 10th plant showed a type of mottling milder than any previously observed. The presence of this extremely mild mottling in one plant suggested that a test be made for possible virus content of the 9 symptomless plants. A transfer was made from each of the symptomless plants to a plant of *N. glutinosa*. Numerous necrotic lesions appeared on three plants, the other 6 transfers causing no symptoms. It, therefore, appeared that a masked infection with a strain of tobacco-mosaic virus had been present in 3 of the Turkish tobacco plants. From the necrotic lesions on *N. glutinosa*, the virus was again transferred to healthy Turkish tobacco plants, and was maintained for a time by serial transfers in that variety of *N. tabacum*. No symptoms were seen in the living plants, although evidence of interference with carbohydrate metabolism in recently infected tissues was found upon staining leaves with iodine. A stock of this masked virus was then derived by transfer from an isolated single lesion on *N. glutinosa* to Turkish tobacco and later to Bonny Best tomato, the juice of which was diluted 1:20 in water and preserved by freez-

ing. The strain of virus represented by this frozen stock will be referred to in this paper as the single-lesion masked strain of tobacco-mosaic virus.

For comparison with the masked strain, and for producing mixtures of known constituents, two other frozen stocks of virus were available. The first was the stock from which the masked strain had been derived; it represented a previously-described (2) single-lesion strain of the ordinary or field-type of tobacco-mosaic virus, hereafter referred to as the distorting strain. The second was a single-lesion stock of an attenuated strain isolated from the distorting-type stock by growth at 34°-35° C., characterized by mottling, but no distortion on *Nicotiana tabacum*, and hereafter referred to

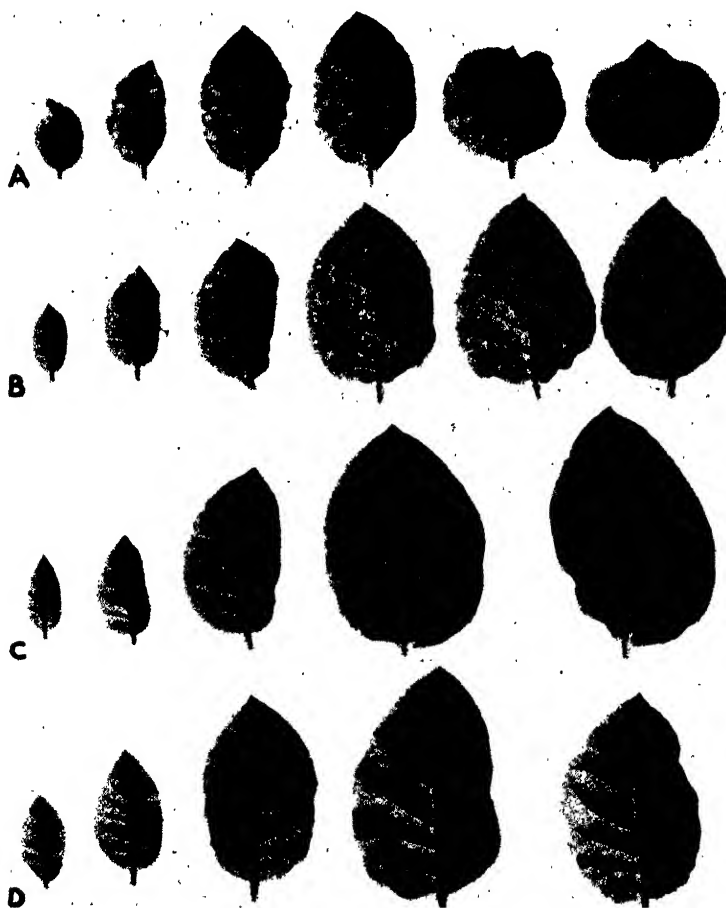


FIG. 1. Successively larger leaves from tops of Turkish tobacco plants 26 days after infection with three strains of tobacco mosaic. A. Distorting. B. Mottling. C. Masked. D. Corresponding leaves of healthy plant of same age.

as the mottling strain. Leaves from the tops of three plants infected with distorting, mottling, and masked strains of tobacco-mosaic virus, and corresponding leaves of a noninfected plant are shown in figure 1.

#### PROPERTIES OF MASKED STRAIN

##### Host Range; Symptoms

The masked strain of virus was used to inoculate a number of species and varieties of plants in order that the resulting infections might be compared with infections by the distorting and mottling strains in the same hosts.

Six species that mottle when infected with the distorting strain were inoculated; they were *Nicotiana tabacum* varieties Turkish, Burley, Connecticut Seed Leaf, Green's Wildfire-Resistant Orinoco, and *chinese*, *N. palmeri* A. Gray, *N. longiflora* Cav., *N. sauveolens* Lehm., *N. sylvestris* Spegaz. and Comes, and *N. trigonophylla* Dun. These six species showed no symptoms with the masked strain, unless a few whitened small veins in some plants of *N. sauveolens* were such; they showed yellowish primary lesions, puckering of young leaves, clearing of veins, and subsequent mottling and distortion with the distorting strain of virus, and yellowish primary lesions, slight clearing of veins, and comparatively early mottling without distortion with the mottling strain. *Solanum nigrum* L. var. *nodiflorum*, which mottles obscurely when infected with the distorting strain, also showed no symptoms when infected with the masked strain.

Three species that respond by mottling preceded by marked stunting and blanching, or by mottling and formation of leafy-outgrowths, or enations, when inoculated with the distorting strain, were tested. The first two of these, *Nicotiana clevelandii* A. Gray, and *N. bigelovii* Wats. varieties *quadrivalvis* Pursh. and *multivalvis* Lindl., which show prolonged stunting and extensive blanching of young leaves when infected with the distorting strain, also showed a tendency toward pronounced symptoms by responding with some distortion to the mottling strain, which does not cause distortion in *N. tabacum*, and with faint but distinct mottling to the masked strain, which produced no obvious symptoms in simultaneous tests in *N. tabacum*. The third species, *N. paniculata* L., which shows enations on the lower sides of severely distorted leaves when inoculated with the distorting strain of virus, demonstrated this tendency to show severe symptoms by a little distortion of leaves in plants infected with the mottling strain of virus and a slight unevenness of color in leaves of plants infected with the masked strain; no enations were caused by the mottling or masked strains.

In all of the above-mentioned host plants, the degree of stunting was correlated with the intensity of chlorosis in the mottling pattern. In gen-



eral, the distorting strain caused relatively severe stunting for a considerable period after infection; the mottling strain caused moderate stunting; and the masked strain caused so little stunting that plants infected with it could not readily be distinguished in this respect from healthy control plants.

Systemic necrosis caused by the three virus strains was studied in *Nicotiana rustica* L. In the relatively young plant, just beginning to form an elongated stem after the rosette stage, the distorting strain caused necrotic primary lesions, severe stunting, puckering of youngest leaves, clearing of veins, early necrosis of small veins in the same area, subsequent death of young leaves and parts of the stem, and finally death of the whole plant. The mottling strain caused similar necrotic primary lesions, but a milder systemic disease with but slight stunting; many necrotic lesions appeared in partly grown leaves, causing leaf distortion, but the plants did not die. The masked strain induced the appearance of similar necrotic primary lesions, but no noticeable stunting; only a few isolated necrotic lesions appeared in partly grown leaves, and these caused little or no disturbance to growth of the infected plant. It appears that the masked strain of virus did not invade the noninoculated part of *N. rustica* nearly so extensively as did the other strains. Symptoms of infections by the three strains could be distinguished from one another in the systemic phase of the disease, but not by the primary lesions, which were approximately equal in number and indistinguishable in size, color, and general appearance.

A number of species in which infection commonly does not become systemic, but that show only necrotic primary lesions as a result of inoculation with the distorting strain, were tested by inoculation with the masked and the mottling strains. These were *Nicotiana langsdorffii* Schrank, *N. acuminata* Hook., *N. glutinosa*, *N. sanderae* Sander, *N. alata* Link and Otto, the F<sub>1</sub> hybrids *N. tabacum* × *glutinosa*, *N. paniculata* × *rustica*, and *N. glauca* R. Grah. × *langsdorffii*, *Capsicum frutescens* L. (necrotic type), *Physalis angulata* L., *Solanum pseudo-capsicum* L., *Datura stramonium* L., and several varieties of *Phaseolus vulgaris* L. In all cases, the necrotic lesions of the three strains appeared alike on any given host species or variety. Necrotic lesions representing the three strains of virus are shown in figure 2 as they appeared on inoculated leaves of *N. langsdorffii*.

One host species that is capable of acting as a symptomless carrier of tobacco-mosaic virus of the distorting strain was tested with the masked strain. This species, *Nicotiana glauca*, always shows mild mottling if infected when very young with the distorting strain; this mottling is often outgrown, with the result that many plants finally become symptomless, although containing virus in some leaves. *N. glauca* showed no symptoms at any stage of growth when infected with the masked strain of virus,

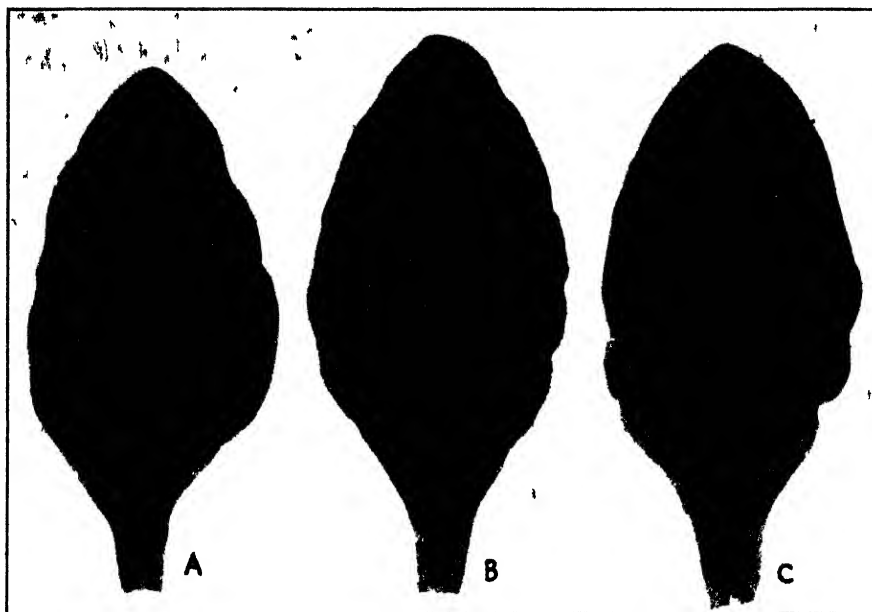


FIG. 2. Necrotic lesions on leaves of *Nicotiana langsdorffii*, inoculated with three strains of tobacco mosaic virus. A. Masked. B. Mottling. C. Distorting.

although virus increased at the site of inoculation and spread to some extent to noninoculated leaves.

To summarize the above observations, it appears (1) that the host range of the three strains of tobacco-mosaic virus was coextensive, so far as tested, no host of one strain having been found immune from another. (2) Primary lesions of necrotic type were indistinguishable in infections by the three strains. Yellowish primary lesions were shown in some hosts by the distorting and mottling strains, but not by the masked strain. (3) All systemic infections with the three strains showed the same sorts of differences. The distorting strain was consistently associated with the most severe stunting, mottling, and distorting, or with the most extensive necrosis; the masked strain induced the least effect; and the mottling strain was associated with symptoms of intermediate type.

#### Starch Retention in Leaves

There were changes in invaded tissues of *Nicotiana tabacum*, in spite of the absence of obvious symptoms. These were changes in carbohydrate metabolism, resulting in starch-retention patterns similar to those previously demonstrated in plants infected with distorting-strain virus (1). By incubation of infected plants for about 16 hours at 10° C. and 4 to 6

hours at 22° C. in darkness, after full sunlight exposure, and by subsequent treatment of leaves with 95 per cent ethyl alcohol to remove chlorophyll, then with 0.6 per cent iodine in 2 per cent potassium iodide solution to stain starch where present, starch-retention patterns were made visible. It was thus possible to show that the masked-strain virus first involved small circular areas in the inoculated leaf. It then affected tissues along main veins and entered such leaves at the top of the plant as would have been affected by clearing of veins in the living plant if distorting-strain virus had been used. The starch-retention patterns at the clearing-of-veins stage were similar in masked and distorting-type infections. In later stages of the disease, however, the masked strain did not appear to invade as extensive areas as were involved by the other strains. The younger leaves of plants infected with the masked strain showed only a few centers of infection in these later stages, as judged by starch-retention lesions. These scattered centers of secondary infection were marked by discrete, ring-like lesions, which were smaller in younger than in older leaves and in basal parts of individual leaves than in apical parts of the same leaves. Figure 3, A, represents leaves from the top of a single plant infected with virus of the masked strain; comparatively few, small lesions of the systemic infection appear in the youngest leaves and younger parts of older leaves. The mottling strain produced more centers of infection in young leaves, but a similar gradient in size of lesions with increasing maturity of leaf tissue. This is represented in figure 3, B, in which leaves from the top of a plant inoculated 12 days earlier show secondary lesions of varying size, in contrast to figure 3, C, in which are shown primary lesions, all of approximately equal size, on similar leaves of a control plant inoculated over all available leaf surface three days before treatment by the iodine-staining method. The fact that lesions develop to approximately the same size in all parts of young leaves inoculated at the same time, but to different sizes in similar parts of leaves infected systemically, suggests that systemic infection of these tissues occurs progressively as they reach a definite degree of maturity. The distorting strain, upon entering young leaves, sets up so many centers of infection in them that separate points of secondary infection could seldom be discerned.

Plants of *Nicotiana glauca* were inoculated with the masked and distorting strains of virus, and their leaves were later stained by the iodine method for demonstrating recently invaded areas of tissue in the systemic infection. The starch-retention lesions in noninoculated, but systemically infected, leaves of young plants of *N. glauca* infected with distorting-strain virus were few. Such as did occur were discrete and ring-like, resembling the starch-retention lesions of the masked strain in *N. tabacum*. In older, symptomless plants of *N. glauca* infected with the distorting strain, the

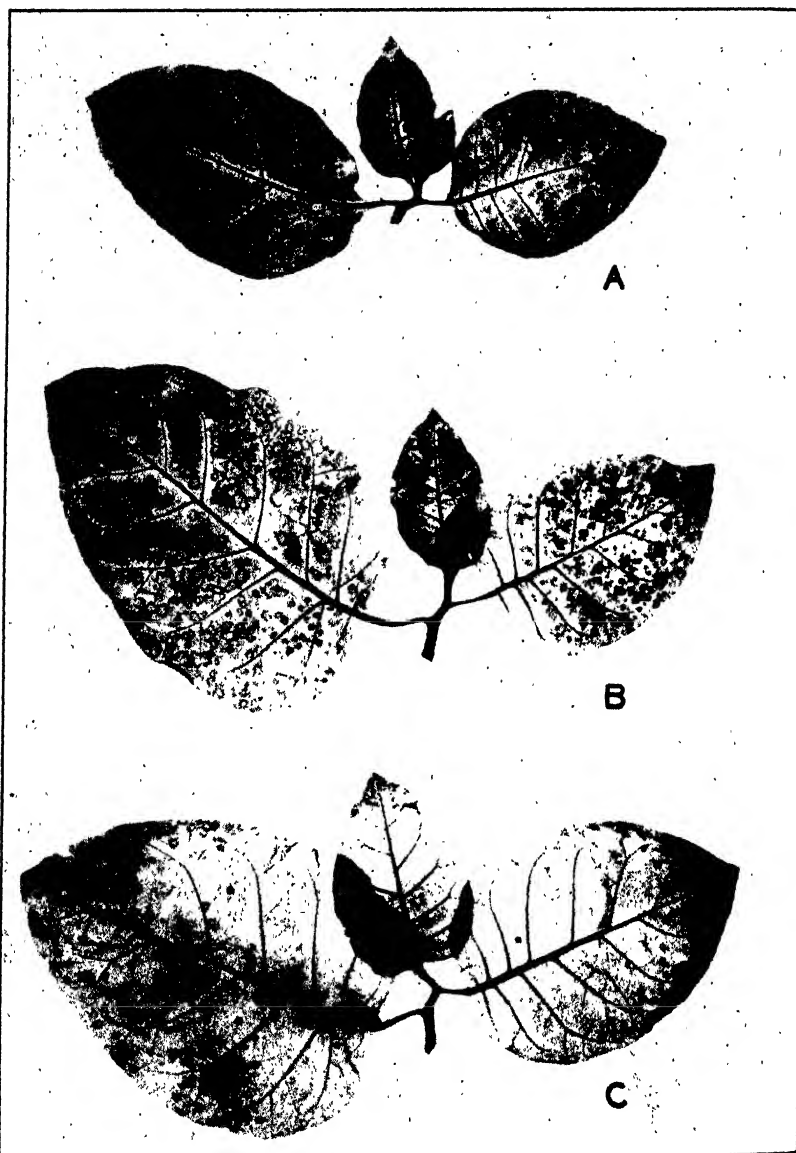


FIG. 3. Leaves stained with iodine to show starch-retention patterns. A. Top of plant systemically infected with masked strain of tobacco-mosaic virus. B. Top of plant systemically infected with mottling strain and showing conspicuous gradient in size of secondary lesions. C. Top of control plant, 3 days after inoculation over whole leaf surface, with mottling strain, showing primary lesions all of approximately equal size.

upper leaves showed even fewer starch-retention lesions. In *N. glauca* plants infected with masked-strain virus, no symptoms were seen in the living plants. After staining leaves with iodine, primary lesions were found to be as numerous as those in infections with distorting-strain virus, but lesions in noninoculated leaves, representing the systemic phase of the disease, were few in number. In some plants no systemic infection occurred.

Failure of extensive invasion of young leaves seems to be characteristic of the symptomless condition in hosts of tobacco-mosaic virus. This was demonstrated and described in an earlier paper (2) as a result of studies of the concentration of virus present in leaves of different ages in plants of the symptomless *Solanum melongena* var. Hangchow Long infected with distorting-strain virus. After publication, it was found that *S. samitwongseii* Craib, another symptomless host of the distorting-strain virus, showed this failure of extensive invasion even more strikingly. In this plant, even long after infection, many leaves near the growing points of branches were almost, or entirely, free of virus; older leaves showed considerably greater concentrations of virus as a result of systemic infection, the oldest leaves being excellent sources of distorting-strain virus. Thus the virus appeared to lag behind the growth of the plant. The present demonstration by a visual method, that is, by staining systemically infected leaves, suggests inclusion of the masked-strain infection of *Nicotiana tabacum* in the same category as infections in symptomless hosts by the distorting strain of tobacco-mosaic virus.

It seems possible that this failure of the masked strain of virus to move freely into young tissues of the plant or to increase much there, may explain in part the symptomless character of the infection. When tissues are finally invaded they may be old enough to be little harmed by the presence of the virus. On the other hand, the fact that primary lesions are not visible on leaves of *Nicotiana tabacum* inoculated with virus of the masked strain, as they are on similar leaves inoculated with the distorting strain, and the fact that the area of clearing of veins, invaded similarly by both strains, is not outwardly affected by the masked strain, indicate also that the presence of virus of the masked strain is less injurious even in invaded tissues.

#### Increase at High Temperature

Preliminary tests showed that the masked strain of tobacco-mosaic virus was able to increase at a temperature slightly higher than the highest temperature previously found suitable for increase of the distorting strain. This was confirmed by simultaneous tests as indicated in the following experiment.

Stems of healthy Bonny Best tomato plants, cut into 3-inch lengths, were divided into three equal sets and inoculated with the distorting, mottling, and masked strains, respectively. Each piece of stem, after inoculation, was dropped into a test tube. The three lots of tubes were then immersed as soon as possible in a thermostatically regulated water-bath and maintained at a temperature of  $35.45 \pm 0.03^\circ \text{C}$ . Tests of concentration of virus in 2 pieces of stem from each lot were made at intervals, using the number of lesions developing on *Nicotiana glutinosa* plants inoculated with juices from the crushed stems as a measure of concentration. The results of the experiment are shown in table 1. The mottling and masked strains of virus produced increasing numbers of lesions in successive tests, but the distorting strain did not. The result of this experiment suggests that the distorting

TABLE 1.—Comparison of ability of three strains of tobacco-mosaic virus to multiply in pieces of stem incubated at  $35.45 \pm 0.03^\circ \text{C}$ .

Interval between stem inoculation and test	Distorting strain			Mottling strain			Masked strain		
	1st test	2nd test	Average	1st test	2nd test	Average	1st test	2nd test	Average
1 day .....	1 <sup>a</sup>	0	0.5	1	0	0.5	10	2	6
2 days .....	0	0	0	6	0	3	25	17	21
3 days .....	0	0	0	0	4	2	43	61	52
5 days .....	1	1	1	61	30	46	165	42	104
7 days .....	1	0	0.5	152	180	166	180	158	169
10 days .....	0	0	0	160	285	222	397	717	557

<sup>a</sup> Figures represent numbers of lesions produced on 5 leaves of *Nicotiana glutinosa* as a result of inoculation with sample indicated.

strain is unable to increase at a temperature at which the attenuated strains increase rapidly.

It might be, however, that at a high temperature the more injurious distorting strain would fail to become established, perhaps killing the first invaded cells or injuring them so as to prevent spread to the remainder of the tissues within the time of the experiment. Ability of the strains to grow at the elevated temperature might be equal if they were once established. To test the possibility that the distorting strain might multiply at high temperature, if once established in the tissues, an experiment was performed in which inoculated stems were not subjected to the higher temperature until 24 and 48 hours after inoculation. Stems of Turkish tobacco were tested for freedom from virus by inoculation of *Nicotiana glutinosa*

plants with juices from part of each. Subsequent failure of the *N. glutinosa* plants to show necrotic lesions was considered evidence that the selected stems were actually not infected before inoculation. The healthy stems were cut into 2-inch lengths, and each piece was inoculated by 30 pin punctures, using as inoculum a single-lesion stock of distorting-type virus, previously stored frozen at 1:20 dilution in water. The inoculated stem pieces were dropped into sterilized bacteriological test tubes. The stems were partly protected from drying by the addition of a short section of younger and noninoculated stem, and by plugging the tubes with cotton. The stems in their tubes were then divided at random into four lots, the first being subjected to a temperature of 21° C. at once, the second to 34.2° C. in a water-bath, the third to 21° C. for 24 hours and then to 34.2° C., and the fourth to 21° C. for 48 hours and then to 34.2° C. Virus increase during ten days was rapid at 21° C., as shown by frequent tests of virus concentration in samples of the stems; it was inhibited completely at 34.2° C. in the sample subjected to this temperature at once, and allowed in a few stems of the samples held for a time at the lower temperature, but finally transferred to 34.2° C. The increase was considerable, however, for only two individual pieces of stem, one incubated 24 hours at 21° C. and then 9 days at 34.2° C., the other incubated 48 hours at 21° C. and then 5 days at 34.2° C. From lesions produced on *N. glutinosa* by inoculation with juices of one of these stems, 34 isolations were made by transfer to young Turkish tobacco plants. All 34 proved to be attenuated strains. A majority of similar isolations from the second stem was also attenuated. The opportunity afforded the distorting-type virus to become established in the cells of the stem before being subjected to high-temperature treatment did not, therefore, allow the unchanged distorting-type virus to increase under these conditions. Later experiments of the same general sort confirmed this result and showed that when any considerable increase of virus occurred in stems subjected to such elevated temperature, even after the distorting-type virus was allowed 48 hours at room temperature to establish itself after inoculation, subsequent isolations on Turkish tobacco showed that the distorting-type virus had been partly or wholly suppressed and some attenuated strain had appeared. The distorting-type virus did not increase at such temperatures, even when an opportunity was given for the virus to become established in the tissues at a lower temperature. The ability to increase at the relatively elevated temperature may, therefore, be considered a differential characteristic of the masked strain of tobacco-mosaic virus described in this paper.

#### Response to Dilution

One of the most striking characteristics of tobacco-mosaic virus is the high infectivity shown when juices of infected plants are diluted with pro-

gressively increasing amounts of water and used as inoculum. Undiluted expressed juices of plants of *Nicotiana tabacum* infected with the masked strain of tobacco-mosaic virus appeared to be nearly as infectious as similar extracts from plants infected with the distorting strain. It was conceivable, however, that progressive dilution with water might bring out some striking difference. Samples were therefore prepared and tested.

Freshly expressed juices of Turkish tobacco plants, infected 25 days previously with masked and distorting strains of tobacco-mosaic virus, were used undiluted and in water dilutions to inoculate *Nicotiana glutinosa* plants. The results, as averages of the number of necrotic lesions on 4 plants at each dilution, were as follows: undiluted samples representing masked strain and distorting strain respectively, 2624 and 2965 lesions per plant (5 leaves); dilutions of 1:10 in water, 1768 and 1930 lesions; of 1:100, 1024 and 1223 lesions; of 1:1000, 499 and 824 lesions; of 1:10,000, 36 and 210 lesions; of 1:100,000, 6.2 and 32 lesions; of 1:1,000,000, 1.5 and 6.8 lesions.

The effect of dilution appears entirely similar for the two strains, both producing moderate numbers of infections when used as inoculum in 1:1,000,000 dilution. The measurements indicated that at all dilutions the masked-strain sample acted as though less concentrated than the distorting-strain sample. This has been observed in other experiments. The observation that iodine-stained leaves show a few scattered secondary lesions instead of numerous ones, such as are characteristic of distorting-strain infections, might explain a lower concentration of virus in expressed juices of plants serving as sources of inoculum.

#### Approximate Thermal Death Point

The masked strain was tested to determine whether its thermal death point was as high as that of the distorting strain, which is outstanding among viruses for its ability to remain infective after treatment at high temperatures. Samples of the masked, mottling, and distorting strains of virus, previously preserved frozen in 1:20 dilutions of juice of infected plants, were thawed and exposed for 10 minutes to temperatures ranging from 70° to 90° C. At each temperature the three strains of virus were exposed simultaneously, being immersed in narrow glass tubes together in a water-bath, the water being agitated by a stirring device. Each sample was tested, after heating, by using it to inoculate two *Nicotiana glutinosa* plants. The results, shown in table 2, indicate that all three samples were reduced to about the same extent by the temperatures 75° and 80° C., and that all failed to infect after treatment at 85° and 90° C. The thermal death point of the masked strain is, therefore, approximately the same as that of the other strains of tobacco-mosaic virus.



TABLE 2.—*Effect of 10-minute exposures of three strains of tobacco-mosaic virus to various temperatures in vitro*

Temperature	Distorting strain			Mottling strain			Masked strain		
	1st test	2nd test	Average	1st test	2nd test	Average	1st test	2nd test	Average
70° C. ....	148 <sup>a</sup>	94	121	54	132	93	19	32	26
75° C. ....	13	2	8	41	22	32	6	3	4
80° C. ....	6	4	5	5	6	6	2	0	1
85° C. ....	0	0	0	0	0	0	0	0	0
90° C. ....	0	0	0	0	0	0	0	0	0
Unheated ..	177	148	162	118	146	132	24	82	53

<sup>a</sup> Figures represent numbers of lesions produced on 5 leaves of *Nicotiana glutinosa* as a result of inoculation with sample indicated.

#### Stability During Successive Transfers in Plants

The stability of the masked strain was tested by 10 successive transfers through sets of Turkish tobacco plants at greenhouse temperature, about 21° to 24° C. Observations were made of the occurrence of occasional aberrant symptoms and of the similarity of plants infected in the final transfer of masked-type virus and plants infected directly from the frozen stock used to initiate the series.

The first infection was obtained by inoculation of 20 plants with frozen stock of juice representing the masked strain. An equal or larger number of noninoculated plants were intermingled with the infected individuals of each set as a control to detect possible accidental infections and to afford healthy foliage for comparison with that of plants infected with the masked strain of virus. Each successive set of 20 or more plants was inoculated from a systemically infected, but not originally inoculated, leaf of one plant of the preceding set.

In the 10th set of the series, 50 infected plants were compared with 50 plants inoculated with the original frozen stock of masked-strain virus. The two sets of plants were kept under close observation. At the end of the first week, noninoculated leaves of 10 plants from each set were tested by inoculation of *Nicotiana glutinosa*; it was found that the masked virus was present in each in spite of the absence of visible symptoms. The infected plants of the 10th set showed no more symptoms than did the plants infected with masked-strain virus from the original frozen stock.

During the course of this experiment, the masked-strain virus was carried serially to 10 sets containing a total of 230 plants of *Nicotiana*

*tabacum*; 390 control plants were used. No symptoms were observed in any of the control plants. Among the 230 inoculated plants, 5 showed visible and hence atypical symptoms; these were mottling patterns, somewhat resembling the usual symptoms of the mottling strain. Fourteen plants showed one or more bright yellow spots; yellow-mosaic strains were isolated from some spots of this type. To this slight extent, at least, the strain was probably unstable. The fact that this strain was capable of being passed through 10 serial transfers at greenhouse temperature without losing its individuality indicates, however, that it is a reasonably stable type of tobacco-mosaic virus.

#### Infectivity After Long Storage

A test of the ability of the masked strain to remain infective after storage was made with dried leaf material and frozen diluted extract of infected plants. This was done to determine whether the masked strain resembled the distorting strain of tobacco-mosaic virus in its property of withstanding long storage. This property of ordinary tobacco-mosaic virus is not shared by certain other mosaic viruses, such as those of cucumber mosaic, and etch of tobacco (4). An example of rapid spontaneous inactivation has recently been described by Samuel and Bald (12, p. 96) who show that the virus of spotted wilt of tomato becomes entirely uninfactive in expressed juice in less than 6 hours at 19° C. and in 3 hours at 22° C.

A fragment of dried leaf in which the masked strain of virus had been preserved from November 23, 1931, to October 13, 1933, was crushed in a sterile mortar with a little water on the latter date. The resulting suspension was used to inoculate plants of *Nicotiana tabacum* var. Turkish and *Datura stramonium*. The infections on *N. tabacum* proved to be typical for the strain, and the necrotic lesions on *D. stramonium* were indistinguishable in appearance and approximately as numerous as control lesions of the distorting strain produced by treating dried leaf material of that strain in a similar way. It is evident that the masked strain of tobacco-mosaic virus is to be considered capable of prolonged storage in dry leaf tissue without becoming inactive, the sample used in this experiment having been preserved for nearly two years before use.

A stock of masked-strain virus was tested after it had been kept frozen two years and ten months in a 1:20 dilution of expressed juice in water. It was found still highly infectious, giving approximately as many lesions on *Nicotiana glutinosa* plants as it had, at the same dilution, when freshly expressed. It produced typical masked infections in plants of *N. tabacum* var. Turkish.

These experiments gave evidence that the ability of ordinary tobacco-mosaic virus to withstand storage for long periods is shared by virus of the masked strain.

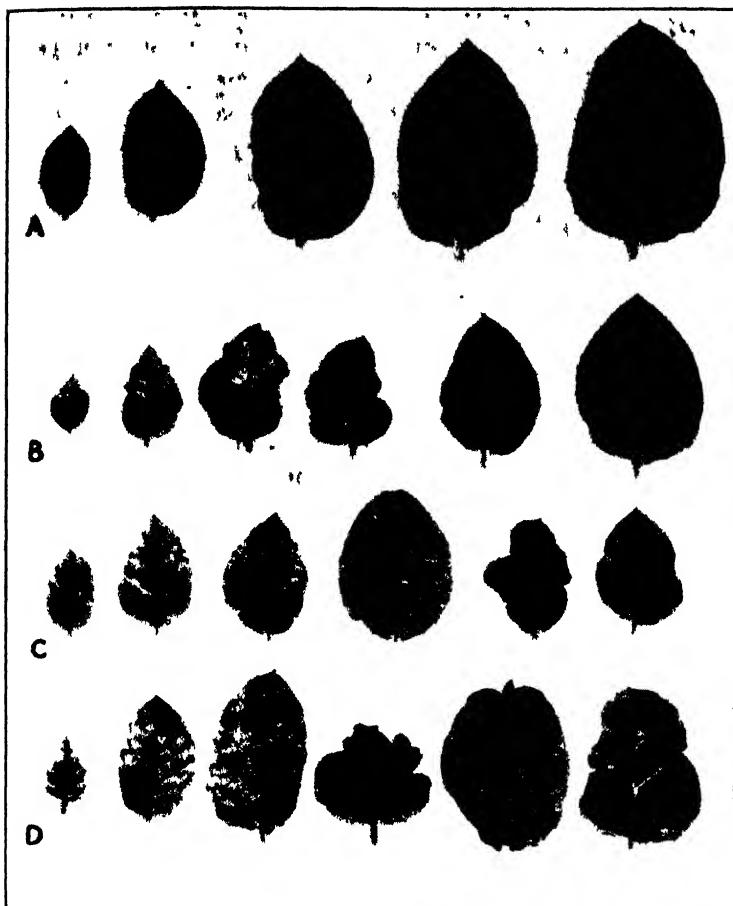


FIG. 4. Successively older leaves from tops of Turkish tobacco plants 26 days after infection with mixtures of strains of tobacco-mosaic virus. A. Masked and motting strains. B. Masked and distorting strains. C. Motting and distorting strains. D. Masked, motting, and distorting strains.

#### Behavior in Mixtures with Other Strains

*Mixture Patterns.* Turkish tobacco plants inoculated with mixtures of strains showed approximately the symptoms characteristic of the more severe strain of the mixture. This is shown in figure 4 (compare with single-strain infections represented in Fig. 1). An additional peculiar symptom, spotting of a leaf originally showing clearing,<sup>4</sup> appeared, however, in many of the plants infected with mixtures of strains. The spotting

<sup>4</sup> The term "clearing" is hereinafter used to designate the symptom previously called "clearing of veins." It is suggested that the longer term is difficult to use in certain phrases, and that the word clearing will prove convenient and distinctive.

became evident after clearing, itself, had nearly disappeared; it was generally conspicuous and erratic in pattern, the spots being characteristically angular rather than rounded. This type of spotting was absent from infections with single-lesion strains of each type. Common forms of mixture patterns and normal clearing in leaves from distorting-strain and mottling-strain infections are represented in figure 5.

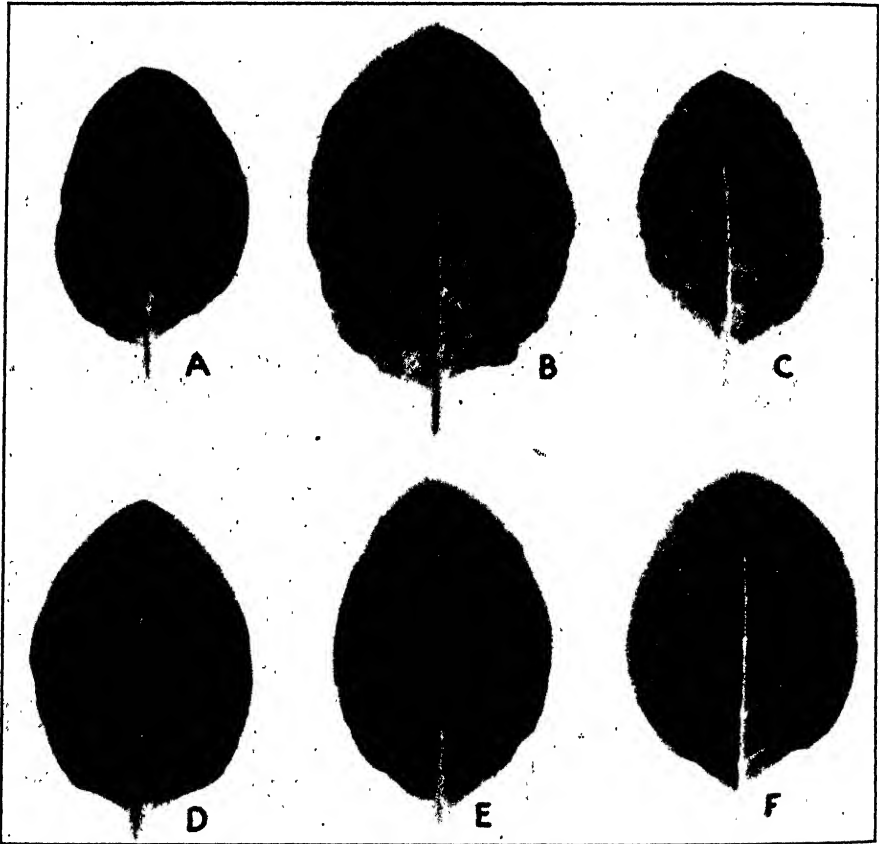


FIG. 5. A-C. Leaves of Turkish tobacco plants showing mixture patterns in plants infected with both distorting and mottling strains of tobacco-mosaic virus. D and E. Normal recovery in leaves previously affected by clearing of veins in plants infected with distorting (D) and mottling (E) strains of virus separately. F. Leaf of corresponding age from healthy plant.

Such spotted patterns never appeared in all plants receiving mixtures of strains. When two simultaneously introduced strains were about equally concentrated, approximately a third of the infected plants showed such mixture patterns. As the amount of either strain in a mixture was de-

creased, the proportion of mixture patterns likewise decreased. When one strain was 1/100 as concentrated as the other, about 5 per cent of the inoculated plants showed mixture patterns. All inoculated plants might reasonably be supposed to receive at least one infective dose of each strain in such mixtures. Failure to show a mixture pattern may be the result of occasional late systemic spread from primary lesions of one of the strains. It has been shown previously that the greater the initial infection with a single strain, the earlier, on the average, the systemic phase of the corresponding disease is manifested (1, p. 298), although a small initial infection may occasionally give an early systemic infection.

Observation of the spotted patterns on leaves that have previously shown clearing is a valuable aid in demonstrating contaminations of one strain with another. It may be used to supplement the methods of detecting mixtures of strains by transfers from single necrotic lesions and by transfers of minimum doses through pin-puncture inoculation.

*Interference with Movement of Other Strains.* The completely masked character of the masked-strain infection in tobacco and tomato suggests the possibility of using the masked strain of tobacco-mosaic virus to protect a plant against later introduction of the ordinary distorting strain of the same virus. Salaman (11) has described such a protective inoculation with the G form of his X virus against the more severe X form. Price (10) has described the modifying effect of ring-spot inoculation on subsequent infection with tobacco mosaic in tobacco. Kunkel (8) has recently described immunity from unattenuated aucuba and tobacco-mosaic viruses in *Nicotiana sylvestris* plants inoculated with attenuated strains derived from each.

Soon after isolation of the masked strain, its ability to protect plants from the effects of infection with distorting-strain tobacco-mosaic virus was tested. Several sets of tobacco plants were inoculated with the masked virus, an equal number of control sets remaining noninoculated. The distorting strain was then inoculated at once into the plants of one set already infected with the masked strain and into the plants of one control set. After intervals of one, two, and three weeks, it was similarly inoculated into other sets. In all cases, symptoms characteristic of infections with the distorting-strain virus finally appeared at the tops of the plants. Interference with the appearance of such symptoms was observable, however, upon comparison of the plants first infected with the masked strain with corresponding plants inoculated with the distorting strain only. In general, plants inoculated with the distorting strain after inoculation with the masked strain showed symptoms later than entirely unprotected plants receiving only the distorting strain. In some cases much more restricted, one-sided infections of the partly protected plants appeared at first, although upon further growth the symptoms characteristic of the distorting

strain always involved the whole top of the plant. The most pronounced action in blocking spread of the distorting-strain virus was found when the masked strain was introduced about one week before introduction of the distorting strain.

This effect of the masked strain in interfering with the movement of a distorting-type strain might, in spite of its incompleteness, be of considerable use in field practice. In the field, primary infections with virulent forms of the virus may be few and late. Inoculation of field plants with the masked virus, with its tendency to make large parts of the tissues of the plants unsuitable for the establishment of distorting-type infections, might reduce injury to the crop by the field type of virus. A preliminary test with tomatoes appeared to support this possibility. A planting of Bonny Best tomatoes in the garden was treated as follows: 23 plants were inoculated with masked-strain virus soon after setting out, 50 plants were inoculated with distorting-strain virus, and 22 were left noninoculated. The fruits were picked early, and weights of green fruit, ripe fruit, and stems with leaves were recorded. The results are shown in table 3. The plants inoculated with the masked strain of virus were distinctly better in average ripe-fruit weight, and slightly better in average yield of green fruit than

TABLE 3.—*Yields of Bonny Best tomato plants inoculated with masked and distorting strains of tobacco-mosaic virus, compared with yields of noninoculated plants*

	Plants exposed only to natural infection	Plants inoculated with	
		Masked strain	Distorting strain
Ripe fruits .....	0.16 <sup>a</sup>	0.29	0.22
Green fruits .....	0.95	0.97	0.87
Stems and leaves .....	0.49	0.54	0.50

<sup>a</sup> Weight in kilograms per plant.

the set inoculated with distorting-strain virus or the set not inoculated at all, but exposed to chance infections in the garden during the growing season. It will be noticed that both inoculated groups of plants showed relatively early fruit ripening, *i.e.*, a large ratio of ripe to total weight of fruit at time of picking (Aug. 27), but that the total fruit weight was low in the plants inoculated with distorting-strain virus. Since no tomato varieties are known to be immune from injury by tobacco-mosaic virus, inoculation with a symptomless strain of this virus at planting time may be worth repeated trial.

*Occurrence of Strains of Tobacco-Mosaic Virus in the Field.* The masked, mottling, and distorting strains of tobacco-mosaic virus are similar

in many of their properties. A distorting type predominates in *Nicotiana tabacum* in the field. This predominance must indicate that the distorting type has exceptional ability to survive competition with other strains in this host.

The survival of the common distorting strain of virus in the field may depend in part upon its ability to spread to and multiply in young tissues. Starch patterns indicate that distorting-strain virus moves into young tissues and produces numerous secondary lesions in the systemic infection; attenuated strains seem to be more limited in their ability to pass into young leaves, apparently entering them later and at fewer points. Failure of the masked strain to protect completely against the distorting strain is perhaps a result of this incomplete occupation of young tissue.

If ability of virus strains to survive in the field is correlated with ability to move into young tissue of the host, one might expect certain plants to exert a filter-like action and to act as selective agents in isolating strains of peculiar properties. Something of this sort seems to occur in wild plants of *Nicotiana glauca*. The strain of tobacco mosaic common on *N. tabacum* tends to be symptomless and erratic in movement in *N. glauca*, as mentioned before. In wild plants of this species such a strain might not be successful because of the tendency of the plant to grow away from the infection. A strain of mosaic virus collected in California in a plant of *N. glauca* growing in an unfrequented spot was transferred to *N. glutinosa* and Turkish tobacco plants in the greenhouse. The necrotic primary lesions on *N. glutinosa* were indistinguishable from those of previously studied tobacco-mosaic strains, but the infections on Turkish tobacco were characterized by unusually pronounced stunting, late formation of new leaves marked by extreme distortion, and the formation of enations, or outgrowths of tissue from the lower sides of the leaves. These symptoms suggest ability to reach very young tissues of *N. tabacum*, because of the marked effect on the growth of the plant and on leaf development.

If this stunting strain enters and occupies very young tissue in *Nicotiana tabacum*, it may be inquired why it does not occur on this host commonly, to the exclusion of the typical field type. The excessive stunting of *N. tabacum* may seriously interfere, however, with chances for spread to other plants of the species. The reason for the dominance of the ordinary type of virus in *N. tabacum* may be that it combines relatively slight stunting of its host with considerable ability to displace slower-moving strains. The most favorable combination of such characters for any particular host would in time determine the common form to be found in that species under natural conditions. Maximum production of infected foliage to ensure frequent transfer from host to host, as well as extensive spread within the host plant to exclude other strains of the virus would be essen-

tial to continued existence. In *N. glauca*, the strain that unduly stunts *N. tabacum* is at no disadvantage, for it produces only mild symptoms and is able to move freely into newly-formed tissues to maintain infection in the individual plant.

ISOLATION OF ATTENUATED STRAINS FROM REPURIFIED STOCK OF DISTORTING-  
STRAIN TOBACCO-MOSAIC VIRUS

Tests of Virus Stocks for Purity

At the time of isolation of the original masked and mottling-type attenuated strains, it was believed that the distorting-strain stock was free of attenuated strains of the virus. Many characteristics of the attenuated strains and adequate techniques for demonstrating them in mixtures were not then known, however. In consequence, it was not possible to attempt to prove that these particular attenuated strains had been derived from virus of the distorting strain, from the stock of which they had been obtained. When study of known mixtures of strains had been completed, it was possible to isolate additional attenuated forms and to investigate the conditions allowing such forms to arise.

The stock of distorting-strain virus from which the first isolation of the masked strain was made had been obtained originally by diluting a supposedly pure, but untested, stock of tobacco-mosaic virus with a million parts of water to one part of juice of a mosaic plant. On 30 plants of *Nicotiana glutinosa* inoculated with this dilution, only 2 primary necrotic lesions appeared. A strain of virus was isolated by transfer from one of these two lesions to a plant of *N. tabacum*. From *N. tabacum*, transfer was made to plants of Bonny Best tomato; 35 days later the tomato plants were crushed and their juices diluted and frozen. When used to inoculate Turkish tobacco plants, this stock showed no indication (as would have been given by the appearance of mixture patterns, had such occurred in tests of the sample) of any admixture as great as 1:100 of an attenuated strain.

From this stock a new isolation was made by a similar process. A 1:1,000,000 dilution of the first single-lesion strain was used to inoculate 20 *Nicotiana glutinosa* plants. A lesion appeared on each of 2 of these plants. The leaf containing one of these lesions was cut into 11 portions, one containing the necrotic lesion, the other 10 consisting of green tissue only. Each of the 10 pieces of green tissue was crushed and the resulting extracts were each used to rub the surface of the leaves of a young Turkish tobacco plant. No disease developed from these inoculations. The 11th portion of the *N. glutinosa* leaf was crushed and transfer was made to 4 young Turkish tobacco plants. Three of these developed typical infections of distorting type. One of them was used as source of inoculum to infect 100 Turkish tobacco plants, the juices of which were preserved frozen.



From a consideration of the method of isolation, which experience has shown to separate two known strains when mixed together, even in equal quantities of expressed juice, it would appear improbable that any attenuated strains originally present in the old stock of virus would have been included in the repurified lot. Repeated tests of the stock immediately after its preservation confirmed this view by showing that mixture patterns were absent from 99 young Turkish tobacco plants inoculated with it. The same stock preserved by (1) freezing and (2) addition of 1 volume of glycerine to 1 volume of the diluted juice was tested again a little later by inoculation of 365 plants with a thawed sample of the frozen juice and by inoculation of 112 plants with the glycerine mixture, but no evidence of the presence of attenuated strains was obtained.

At the time of repurification of this stock of distorting-strain virus, a stock of tobacco-mosaic virus that had been kept frozen for about 8 years was found to give many mixture patterns. Upon minimum inoculation of a series of plants it was found to be a source of many attenuated strains, as well as of the supposed original distorting strain. This old stock did not furnish any completely masked strains, but about one-third of the isolations resembled the mottling type described in this paper, or were intermediate between this type and the distorting type. Naturally, nothing was known about the original purity of this stock, although it presumably represented typical tobacco-mosaic virus when first preserved. The source of the numerous attenuated strains obtained by direct minimal inoculation from it after it had been frozen about 8 years was, therefore, a matter of great interest, since the method of preservation might be considered to have induced their formation, and since the stocks that had been purified might also be considered in danger of spontaneous conversion to attenuated types after a time.

Aging of the frozen stock might have produced such attenuated strains. Tests of the first distorting-strain stock that had been carefully isolated were made. At the time of testing, this stock had been frozen  $3\frac{1}{4}$  years. Two hundred young Turkish tobacco plants were inoculated by 5 pin punctures each, using this stock virus; 140 plants remained healthy, 60 showed distorting-type symptoms. Thus this stock of distorting-strain virus, after being frozen for more than 3 years, showed no evidence of admixture of attenuated types. No older, properly purified, frozen stock of virus was available. This same stock of virus, when it had been preserved frozen for 2 years, had been mixed with glycerine (10 volumes of glycerine to 1 volume of thawed undiluted stock of distorting-strain virus, and 9 volumes of water) and soon afterwards tested for purity of the distorting-strain virus by inoculating 321 Turkish tobacco plants. No evidence of admixture of attenuated strains in this stock had been obtained at that time. The

results of these experiments bring evidence that storage of virus in a frozen condition for two or three years does not induce the formation of detectable quantities of attenuated strains.

The original method of securing the impure stock might have been responsible for the occurrence of the attenuated strains. This stock had been obtained from old plants instead of from comparatively young ones, such as had been used for all of the more recently purified and tested stocks. The repurified stock was, therefore, introduced into young Turkish tobacco plants, and tests were made of the purity of the strain in these plants 4, 8, 13, 16, and 21 weeks after inoculation. On each occasion 100 to 200 healthy young plants were inoculated from various parts of the infected plants in the attempt to detect the presence of newly formed attenuated strains. At no stage was the presence of attenuated strains demonstrated, although all leaves, as well as stems, blossoms, and seed pods, had been included in the tests.

A little evaporation undoubtedly had occurred in the 8-year-old frozen stock of virus. Some residue, containing virus, must have been left on the sides of the tubes. This exposure of virus in dried condition might have induced the formation of attenuated strains. Dried leaf material of purified stock, about  $3\frac{1}{2}$  years old, was ground in a little water, therefore, and tested for purity of the distorting-type virus contained therein. Turkish tobacco plants were inoculated by pin punctures, 5 to each plant. Among 360 inoculated plants, 103 distorting-type infections and 2 possibly attenuated infections were secured. A set of 128 similar plants, inoculated by rubbing, gave 121 typical distorting-type infections and 7 suspected mixture patterns. The 9 aberrant infections were transferred repeatedly to observe symptoms and isolate any attenuated strains. One of the two possibly attenuated strains proved to be the typical distorting strain, perhaps expressing its symptoms at first in an abnormal plant; the other appeared actually different from the distorting strain, but only slightly so, giving some distortion. From transfers of the 7 suspected mixture patterns, a single case of a definitely attenuated strain was finally isolated; this result suggests that drying for  $3\frac{1}{2}$  years may have been effective in modifying the type.

The evidence for attenuation by drying seems hardly sufficient to account for the high proportion of attenuated strains in the 8-year-old stock; the origin of these strains, therefore, remains unexplained, since the more recently isolated frozen stocks derived from single lesions did not show evidence of detectable contamination of this sort.

#### Repeated Production of Attenuated Strains at High Temperature

Familiarity with the behavior of known mixtures of strains had made it possible to test the supposedly pure-strain frozen stocks, and it had been

found that these acted as though free from any appreciable contamination with attenuated strains. On the other hand, none of the tests could be expected to exclude the possibility that extremely small amounts of contamination might exist in such apparently pure stocks. It seemed necessary to repeat the work of isolation of attenuated strains in a quantitative way, to determine whether such strains could be isolated frequently after minimal inoculation.

In the following experiment an attempt was made to secure as many separate instances of attenuation as possible from a relatively few initial infections. About 120 pieces of stem, each about  $1\frac{1}{2}$  inches long, were cut from healthy Turkish tobacco plants. They were handled only with virus-free instruments and materials. A sample of each stem was tested by inoculation of *Nicotiana glutinosa*; no lesions appeared as a result of these inoculations, indicating that the stems were actually free from virus. The pieces of stem were inoculated with a 1:20 dilution of distorting-strain tobacco-mosaic virus, frozen since repurification. Five pin punctures were made in each stem, the pins being wet with inoculum before inserting them into the tissues. The inoculated stems were dropped into sterile test tubes, which were then stoppered lightly with cotton. These tubes were divided into two lots by putting alternate tubes into different wire baskets. All the tubes and their inoculated stems were held at a temperature of approximately  $24^{\circ}$  C. for 24 hours, before any were exposed to a higher temperature. This was done to aid in establishment of the minimal infections that had been given by the pin-puncture inoculation. Failure to allow some opportunity for establishment of virus at a temperature lower than  $34^{\circ}$  to  $35^{\circ}$  C. had been found to cause complete inhibition of all infections in certain earlier experiments. At the end of 24 hours, one lot of stems was allowed to remain at  $24^{\circ}$  C., the other was transferred to a water bath at  $34.2^{\circ}$  C. In order to improve the chances of establishment of all infections, the tubes exposed to high temperature were removed from the water bath at intervals during the first few days, being held at  $24^{\circ}$  C. with the control tubes for 6 hours on the third day, 7 hours on the fourth day, 5 hours on the fifth day, and 6 hours on the sixth day, as well as for the whole 24 hours of the first day after inoculation. Except for these periods, the high-temperature set of tubes was maintained at  $34.2^{\circ}$  C. in the water bath continuously until the eleventh day. It is not known that this program of alternating temperatures is essential, or the best one, but it did give the desired effect of establishing approximately the same proportion of infections in the stems treated at high temperature as in the control stems, as later test showed.

At the end of 11 days of incubation, the stems of the control set were crushed and their juices used to inoculate *Nicotiana glutinosa* plants. From

the resulting infections it was found that virus had multiplied at 24° C. in 55 of 60 inoculated stems. Thus, not less than 55 minimal infective doses of virus could have been concerned, since 55 separate stems became infected. If the assumption were made that the distribution of the primary infections among the 60 stems were by chance alone, a total of 168 minimal infective doses would have been the most probable number to have caused the 55 infections in 60 inoculated stems. Since any variability in stems or inoculation technique would have tended to increase this figure rather than to decrease it, it seems reasonable to assume a large number; it is judged that at the most several hundred minimal infective doses may have been introduced into this set of stems.

The stems of the set exposed to the higher temperature of 34.2° C. for a total of 9 of the 11 days following inoculation were also crushed individually and their juices used to inoculate *Nicotiana glutinosa* plants. From the resulting infections it was found that virus had multiplied in 49 of 55 inoculated stems of this set; this incidence of infection (89 per cent) was almost exactly the same as had been found among the controls (92 per cent). It may be assumed that all of the stems had originally received as great an average infection at time of inoculation as had the stems of the control set; therefore, it may be considered that several hundred minimal infective doses of virus were introduced into this set also.

Some of the primary necrotic lesions on the 49 *Nicotiana glutinosa* plants, infected from the set of stems incubated at 34.2° C., were used as sources of inoculum for transfers to young Turkish tobacco plants, 5 lesions from each original stem being used at first in most cases. Each lesion was ground separately and the resulting fluid was used to inoculate a single young plant of *N. tabacum* var. Turkish. Of the 49 sets of Turkish tobacco, each of 5 plants, all but 13 sets showed one or more infections with attenuated strains of tobacco-mosaic virus. An attempt was made to repeat the transfers for the 13 series that had yielded no attenuated strains, using additional necrotic lesions as sources of virus for test inoculation. From each of the 13 appropriate *N. glutinosa* plants additional necrotic lesions were isolated and crushed, and the juices were used as inoculum on additional young Turkish tobacco plants. Eleven days later it was found that 10 of these 13 new series showed one plant or more infected with an attenuated strain. By this time the original *N. glutinosa* leaves were not suitable for further use, and the three series that had yielded no attenuated strain were abandoned. Thus, of the total of 49 stems in which attenuated strains could have been produced, 46 were actually demonstrated to have produced an attenuated strain. Some of the series had produced more than one type of attenuated strain, many types from nearly masked to clearly mottling appearing in this experiment. The number of times that an at-

tenuated strain was produced from the several hundred original infections derived from the stock of repurified distorting-strain virus was in consequence more, rather than less, than 46.

#### Retesting of Stock; Absence of Attenuated Strains

The results of earlier study of the stock of distorting-strain virus had indicated that 46 or more isolations of an attenuated strain could not be expected in a few hundred minimal infective doses demonstrated by direct inoculation from it. In view, however, of the ever-present possibility that the passage of time might affect the purity of the frozen stocks, it seemed desirable to retest the particular stock used in this experiment and compare it with known mixtures of strains after completion of the attenuation experiment.

The thawed sample of virus stock prepared for the original stem inoculation in this experiment had been divided into two portions, and one of these had been refrozen as a control on the continued purity of the stock virus, the other having been used in the inoculation of stems. When the results of the experiment were known, the refrozen portion was thawed and tested to determine whether it was still free from attenuated strains. Successive tests were conducted as follows.

Young Turkish tobacco plants were inoculated with the residue of the 1:20 stock on three occasions, the inoculum being thawed and refrozen each time. Each plant was punctured with a set of 5 insect pins inserted near the base of a young leaf. In the first set 100 plants were inoculated, 42 showed symptoms typical of infection with the distorting strain, none showed mild-mottling symptoms. In the second set 150 plants were inoculated, 63 showed distorting-type symptoms, none showed mild symptoms. In the third set 150 plants were inoculated, 81 showed distorting-type symptoms, but none showed mild symptoms. Thus, in 400 inoculated plants, 186 typical infections of the distorting type arose, but no attenuated strain was found.

As a control, a mixture was made of 10 parts of the 1:20 stock distorting-strain virus with 1 part of the 1:20 mottling-strain stock. In a set of 100 Turkish tobacco plants inoculated with this mixture, using 5 pin punctures in each plant as before, 51 plants remained healthy, 46 plants showed distorting-type symptoms, 2 plants showed mottling of the mild type without leaf distorting, and 1 showed a mixture pattern, indicating infection with both strains of virus. The success in isolation of an attenuated strain from a 1:10 mixture by this method of minimum inoculation indicated that no contamination of the original stock could have approached this 1:10 ratio. It will be realized that this method of minimum inoculation could not be used to demonstrate the presence of an attenuated strain of virus in

smaller ratios to the distorting-strain virus, as in the ratios 1:100 to 1:1000, without the use of very large numbers of test plants. A more sensitive test of the presence of admixed attenuated strains was at hand, however, in the form of the test for the production of mixture patterns.

The mixture-pattern test was applied to a portion of the original stock refrozen after the attenuation experiment was started. One hundred Turkish tobacco plants were inoculated with the supposedly pure distorting-strain stock. These plants were rubbed with a glass spatula to give a heavy inoculation from the 1:20 dilution of juice. Another 100 plants were inoculated similarly, but with 1 part of 1:20 mottling-strain stock purposely mixed with 1000 parts of the 1:20 distorting-strain stock (proportion of strains, 1:1000). A third 100 plants were inoculated with a 1:100 mixture; a fourth set involved a 1:10 mixture. A fifth set of a hundred plants was reserved for inoculation with the mottling-strain stock alone. As a result of the inoculation, all plants except one of the mottling-strain set became infected. It is believed that this plant may have been left uninoculated. At the end of two weeks, the first set showed no mixture patterns, but 100 typical distorting-type infections; the second showed 19 mixture patterns; the third and fourth sets showed 5 and 1 mixture patterns, respectively; the fifth set showed no mixture patterns, nor distorting-type infections, but all mottling-type infections, except for the one plant that appeared to have escaped inoculation. Evidently the residue of the original stock of distorting-type virus used in the attenuation experiment had not contained so much attenuated virus as did a purposely made mixture of 1 part of mottling-strain stock in 100 parts of distorting-strain stock.

#### DISCUSSION

With present methods it is not feasible to demonstrate that very small traces of attenuated strains are not present in such a stock as the supposedly pure distorting-strain stock described in this paper. Evidence has been given, however, for believing that the distorting-strain stock did not contain so much of attenuated strains as would a mixture of a hundred parts of this stock and one part of a mottling-type stock of similar virus concentration. The isolation of at least 46 attenuated stocks from a few hundred minimal infective doses derived from the original distorting-strain stock of tobacco-mosaic virus furnishes some evidence, therefore, for the contention that the appearance of attenuated strains of tobacco-mosaic virus during incubation of the ordinary distorting strain in plant tissue at a temperature of a little above 34° C. is not to be ascribed to selection of some contaminant in the original virus stock but is a result of the action of high temperatures on the distorting-strain virus. It is not contended that failure to find such derivatives in purified stocks of the distorting strain as a result

of exposing them to freezing or long standing in tissues of living plant hosts indicates that attenuated strains do not sometimes appear under these conditions. The experiments herein reported have merely shown that the distorting strain of tobacco-mosaic virus is relatively stable under these conditions, yet readily produces attenuated strains during incubation in living tissues at temperatures just above 34° C., as originally indicated by Johnson (5) in 1926.

The identity of host range, so far as tested, and constant relationship of symptoms of the distorting, mottling, and masked stocks of tobacco-mosaic virus are striking. This confirms the evidence for close relationship of these strains to each other, as given by the experiments concerned with the derivation of one type from another. Other viruses, such as those of cucumber mosaic, mottle of potato, bean mosaic, raspberry mosaic, etc., are not known to show corresponding similarities over an extended host range and are believed not to be closely related to each other or to tobacco-mosaic virus. It seems appropriate to refer to such stocks as have no observable similarity in symptoms over an extended host range as separate viruses, but to those in which an evident relationship of symptoms occurs over an extended host range as strains of a single virus. This practice has been followed in the present paper. The intended distinction is comparable to that between species and varieties among plants.

The clearly recognizable characteristics of the three strains of virus described in this paper may lend assistance in the study of the fundamental question of virus nature. Accumulated evidence concerned with the behavior of tobacco-mosaic virus is interpreted by some as indicating that the virus is of the nature of an organism, by others as indicating that the virus is a single chemical compound. It is, of course, also possible that the virus may represent a primitive, or a degenerate, obligate parasite, consisting of an organized group of a relatively small number of chemical compounds. Whatever the nature of the virus, whether a complex living organism, a single chemical compound, or a small organized group of chemical compounds, the study of the conditions allowing the transmutation of one strain to another may prove to be important. It has been shown that many properties of the three strains of tobacco-mosaic virus described in this paper are alike for all strains, and that there are a few distinctive properties. If the virus were a chemical compound, the strains would necessarily represent related new compounds or isomers. If a chemical treatment could be found that would consistently change one of these strains into another, it would probably be feasible to develop the quantitative study of the reaction because of the opportunity to estimate the concentration of each strain. If the virus were a primitive or a degenerate organism, the possibility that a simple relationship, even a simple chemical relationship, might exist between the

strains is not entirely excluded. It is, indeed, among the viruses, if anywhere, that one might look for living matter so simple in constitution that its constituent materials might be few enough to be readily studied.

The difficulty of invasion of very young tissues by the masked strain of virus raises an important theoretical consideration. The difference in fundamental nature of the masked strain and distorting strain may be of such a sort as to make it easier for the plant to restrain movement of masked-strain virus within leaf tissues. There is a possibility that study of this characteristic of the masked strain may later add to our knowledge of virus nature some information on the particular way in which these strains differ from each other.

#### SUMMARY

A completely masked strain of tobacco-mosaic virus, possessing many properties of the ordinary distorting, field-type virus, was studied in comparison with a typical mild-mottling strain and with the distorting strain from which it appeared to have been derived. Mixtures of the distorting strain and the masked and mottling-type attenuated strains could be distinguished with ease from single-lesion, presumably pure, stocks of the distorting strain when as little as one per cent of the admixed type was present. It was shown that stocks of distorting-strain virus, acting as though not containing admixtures to this extent and presumed to be entirely free from attenuated strains, remained free from such detectable admixtures of mild-mottling types in plants and in frozen juice for considerable periods of time. Small inoculations of such stocks of distorting-strain virus into healthy stem tissues incubated at temperatures a little above 34° C. gave rise frequently to attenuated strains. The evidence points to the appearance of new and stable strains from the old stock.

The differences found between the original and the derived strains were few in comparison with their common properties. The masked, mottling, and distorting strains were similar in host range, approximate thermal death point, ability to remain infective after long storage, infectivity in undiluted and diluted juices, production of necrotic primary lesions identical in size, form, and general appearance in certain hosts, and starch-retention patterns in inoculated leaves of certain other hosts. The strains differed principally in two respects. The attenuated strains were able to increase in host tissues at temperatures high enough to inhibit multiplication of virus of the original distorting strain. They caused less chlorosis in the mottling patterns and appeared to involve tissues near the growing point later than did the typical distorting-type tobacco-mosaic virus. In the latter respect the relation between the masked strain and *Nicotiana tabacum* resembles the relation previously shown to exist between the distorting strain



of tobacco-mosaic virus and its symptomless carriers, which do not show yellowish primary lesions at the site of infection and which do not allow this strain to enter, or else do not allow it to come to high concentration in, cells of young tissues.

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# THE CYTOLOGY OF *UROCYSTIS OCCULTA*<sup>1</sup>

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## INTRODUCTION

As part of a comprehensive investigation of *Urocystis occulta* (Wallr.) Rabh., the writers studied the cytological phenomena as a basis for a study of the possible origin of races through hybridization.

The cytology of several species of *Urocystis* has been investigated by several investigators, notably Paravicini, Kniep, Rawitscher, and Blizzard.

Paravicini (4) studied *Urocystis anemones* (Persoon) Winter and *U. violae* (Sow.) Fischer v. Waldh. According to him, long, several-celled mycelial threads are abjointed from the tips of promycelia of *U. anemones*. The cells of these threads are uninucleate and the dikaryophase arises from the fusion of neighboring cells in the same mycelial thread. Paravicini states that the fusions were difficult to see and that there probably are other types of fusions, also. According to his observations, the sporidia of *U. violae* are readily abjointed from the promycelium and then fuse in pairs. Kniep and Rawitscher, however, do not agree with Paravicini.

Kniep (2, 3) investigated *Urocystis anemones*. He illustrates a short promycelium with several uninucleate sporidia, which do not become abjointed. The sporidia fuse in pairs at the base, the nucleus from one passes into the other, which then elongates into a binucleate hypha, thus initiating the dikaryophase.

Rawitscher's observations on the origin of the dikaryophase in *Urocystis violae* agree with those of Kniep on *U. anemones*. Rawitscher (5) states that the origin of the binucleate condition was hard to determine, because 8 sporidia usually are formed and are so crowded at the base that the fine fusion tubes are difficult to see. He states that the binucleate sporidia may become abjointed from the promycelium and bud, the 2 nuclei undergoing conjugate division. In this respect, then, *U. violae* apparently is different from *U. anemones*.

Blizzard (1) found no evidence of fusion between promycelial branches of *Urocystis cepulae* Frost. These branches may become multicellular, but each cell contains a single nucleus. Blizzard states that the saprophytic mycelium "is uninucleate from the beginning and remains so throughout

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its existence." He states that the cells of the parasitic mycelium also are uninucleate at first but "become progressively binucleate as they approach the young sorus primordium, where all the cells of the sporogenous hyphae are binucleate."

On the basis of these reports there appears to be considerable variation and variability between and within these 3 species. The observations of Kniep and Rawitscher agree in essentials. The apparently conflicting results of Paravicini and Kniep on *Urocystis anemones* may be due to differences in conditions under which spores were germinated, although both authors agree that fusions occur and are sexual in nature. Blizzard's observations in *U. cepulae* appear to be an exception to the widely accepted view that the fusion of sporidia or promycelial cells initiates the dikaryophase. It is well known, however, that the dikaryophase of most smuts does not maintain itself well on artificial media; hence, one would expect that *U. cepulae* might be in the haplophase when grown on onion agar, as Blizzard found it to be. The lack of fusions between promycelial branches might be accounted for in the same way, although there is no reason why all species of *Urocystis* should necessarily behave alike in this respect. A comprehensive study of different species under various comparable conditions is desirable.

#### SPORE GERMINATION

Wolff (6), as early as 1873, described and illustrated spore germination in *Urocystis occulta*. He states that the spores require from 3 to 4 days to germinate, that they send out a promycelium through a split in the wall, and that 2 to 6 sporidia are formed. He noted, further, that very rarely certain sporidia were united by "eine Art von Brücke"—a kind of bridge; and he figures a distinct H-shape fusion, but does not show the nuclear condition.

In general, the writers' observations agree with those of Wolff. In our experiments the spores germinated best after having been soaked for about 16 hours in water to which benzaldehyde had been added at the rate of 3:2,000,000. Germination tests were made at various temperatures, ranging from 9.5° to 28° C. Early germination was best at about 15°, but 24° was more favorable for subsequent development. Details of the relation of temperature to spore germination and the development of smut will be discussed in a separate paper.

Under favorable conditions spores usually germinate well in from 30 to 48 hours. The spore wall is split and a promycelium is sent out. It may be short and nonseptate or may grow to considerable length without forming cross walls. Sometimes, however, it becomes septate and may consist of as many as 10 to 15 cells, the protoplasm usually being aggregated in the tip cell.

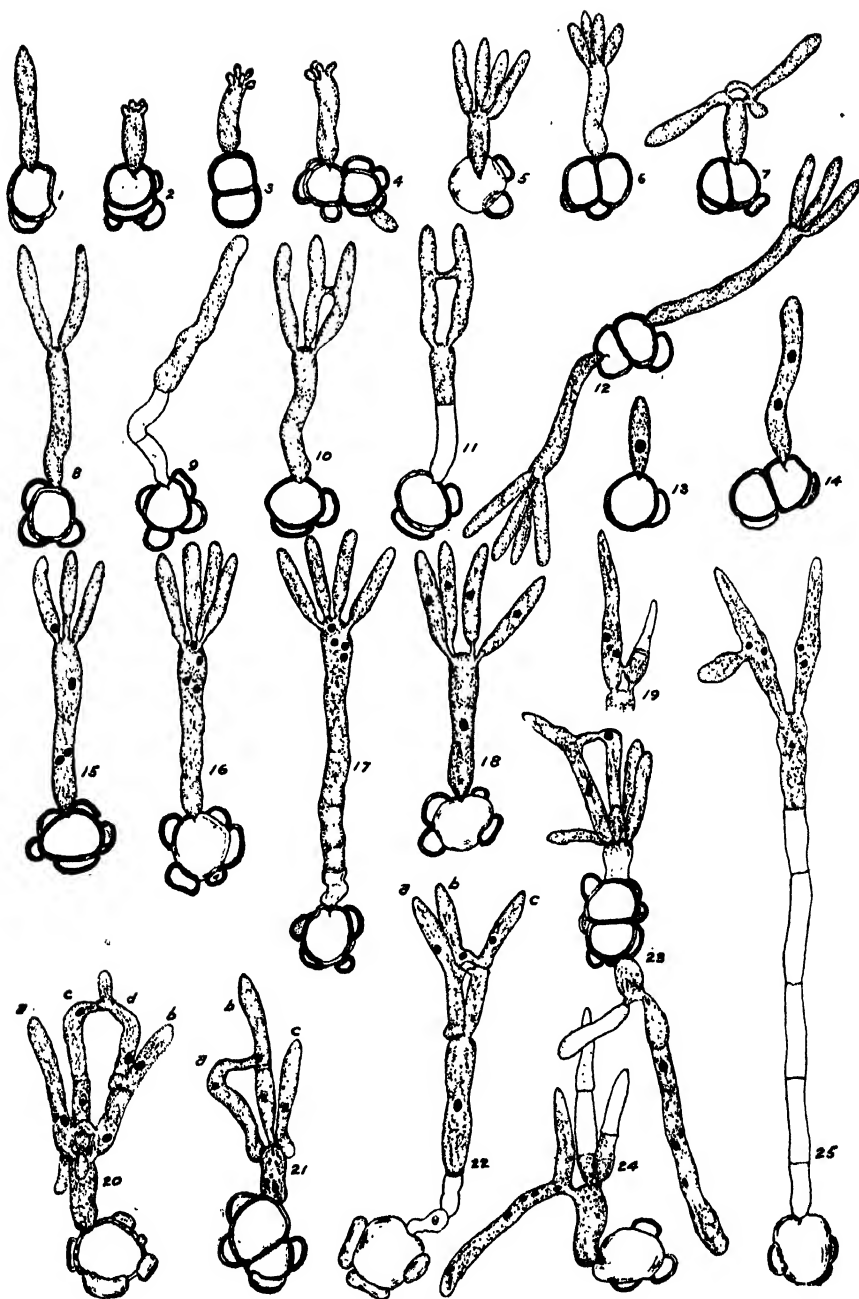


PLATE I

Sporidia begin to form within a few hours after germination. They are somewhat variable in size and shape, and quite variable in number and in time of formation. Often they begin to form when the promycelium has attained a length of only 20 or 30  $\mu$ , but they may not begin to develop until it is many times as long (Plates I and II). The number ranges from 2 to 6, 4 being a very common number. Sometimes there appear to be still more, but in such cases some are small and possibly should not be considered as sporidia. Relatively short, thick protuberances often grow out from the base of the whorl of sporidia, either from the promycelium itself or from the base of the sporidia.

The sporidia, as far as we have been able to observe, remain attached to the promycelium and do not function as conidia, although further study

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PLATE I

Stages of spore germination of *Urocystis occulta* in water. 1-6. Young promycelia showing the formation of sporidia. 7. Basal fusion between two sporidia. 8-9. Later stages. 10-11. II-shape fusions between sporidia. These are fairly common. 12. Germination of a 2-celled spore, each fertile cell of which has sent out a rather long promycelium.

Development on oat-meal agar. 13-14. Young promycelia, showing a single large nucleus in 13 and two nuclei in 14. 15-17. Promycelia on which the sporidia are completely or almost completely formed; nuclei about to pass into the sporidia. 18. Each sporidium has received a single nucleus, and there is one in the promycelium. 19. Fusion between two sporidia on the promycelium. The nucleus from the one on the right has passed to the sporidium on the left, which has begun to elongate, while the one on the right is becoming vacuolate. 20. Fusions between sporidia. Sporidia *a* and *b* have fused near the base; *b* has become septate, each cell containing a nucleus; the upper cell is sending out a protuberance that probably will fuse with the lower cell in *c*. The tips of sporidia *c* and *d* have fused, and a hypha is being sent out from *d*, into which the nucleus from *c* is moving. Sporidium *c* has become 2-celled, each cell with a single nucleus. The basal cell appears to be sending out a protuberance near the septum, preparatory to fusion with the upper cell of *b*. Short, bulbous branches, such as those shown at the base of the sporidial whorl, are fairly common. 21. Sporidia *a* and *b* have fused and *b* is beginning to elongate. A septum has been laid down just below the fusion tube in *b*. The lower cell contains a nucleus and appears to be about to fuse at the base with *c*. There appear to be two nuclei in the promycelium. 22. Sporidia *b* and *c* have fused. The nucleus from *b* is passing into *c*, which has formed a cross wall just below the fusion tube, and the lower cell is about to fuse with sporidium *a*. 23. Two sporidia on the upper promycelium have fused and the one on the left is beginning to send out a hypha. Evidently, fusion has already occurred on the lower promycelium, but it could not be seen. 24. Four nuclei from the promycelium apparently have passed directly into the hypha growing out from the sporidium on the left. There is no evidence of fusion, and the two sporidia on the right are degenerating. Apparently the fungus has taken a short cut in initiating the dikaryophase, and the nuclei have become paired without previous fusion between sporidia. 25. A long, septate promycelium, the protoplasm aggregated into the tip cell on which each of the two large sporidia contain two nuclei, presumably derived from the promycelium directly, as there is no evidence of fusion.

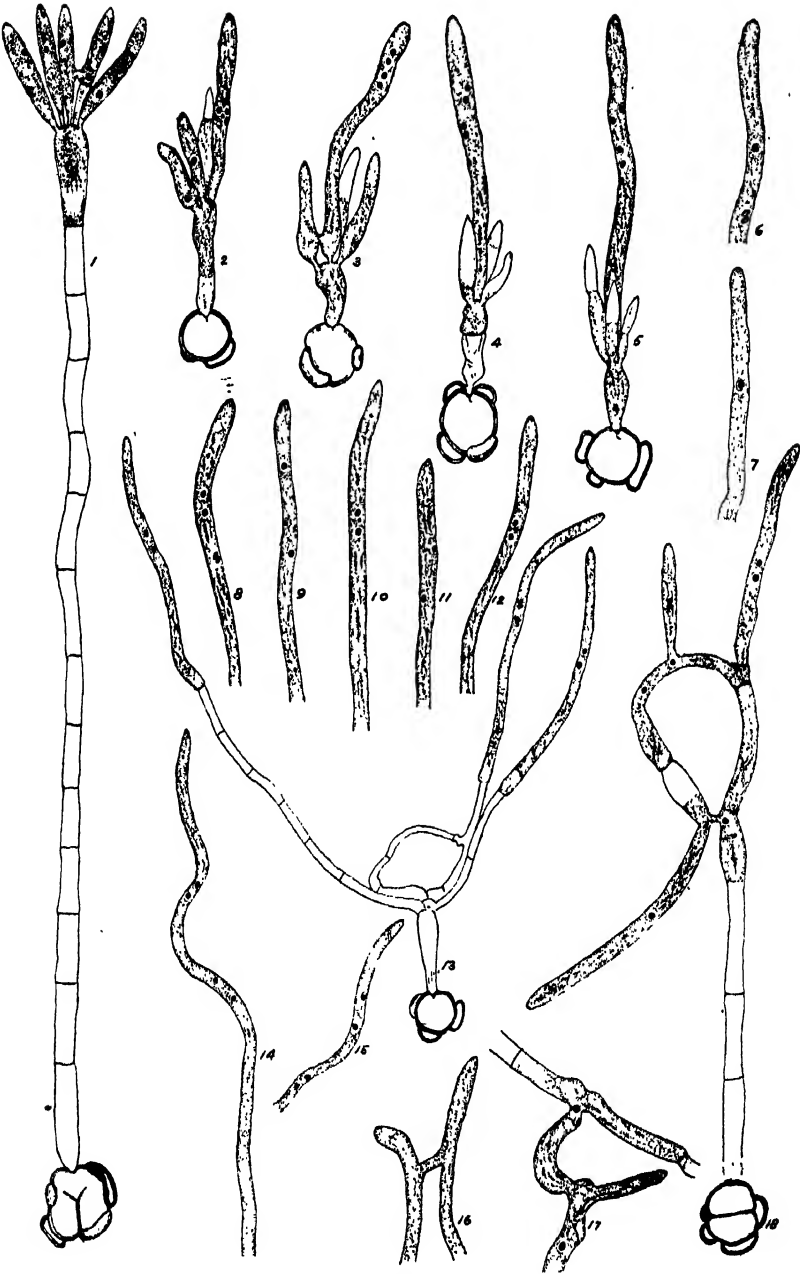


PLATE II

of their behavior under different conditions is desirable. Apparently, however, they represent a greatly shortened haplophase in the life cycle of the organism. They grow, receive a haploid nucleus from the promycelium, fuse with another sporidium, and either degenerate or send out a dikaryotic hypha, depending on whether they are on the delivering or receiving end of the fusion tube. If they do not fuse, they seem to degenerate in most cases. The haplophase, therefore, seems to be limited in function as well as in duration, under the conditions of the writers' experiments.

#### NUCLEAR BEHAVIOR

*Germinating Spores.* Spores were dusted on a thin film of oatmeal agar on slides, which were then placed in a moist chamber until the desired stage of development of the fungus had been reached. The material was then killed in Flemming's weaker solution, washed and fixed, and stained with iron-alum haematoxylin.

The normal nuclear behavior may be summarized as follows. The young promycelium contains a single, large nucleus, in which a nucleolus usually is clearly visible. Successive nuclear divisions occur, resulting in the for-

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#### PLATE II

The development of the dikaryophase. 1. A somewhat unusual type of promycelium, comprising many empty cells and one cell at the tip into which the protoplasm has been aggregated. Two of the sporidia have fused. 2-5. Early stages in the development of dikaryotic hyphae, such as result from the fusion of sporidia. In 2 the sporidium, which is becoming vacuolate, evidently has fused with the one that is elongating into a hypha, although the fusion tube was not visible. In 3 a fusion tube is clearly visible. In 4 and 5 a single hypha has been sent out in each case, and the other sporidia are degenerating. The nuclei are arranged in two pairs. 6-12. Hyphal tips, such as those shown in 13. In 6 there are three nuclei. Apparently the lower pair have just resulted from division, while the upper nucleus has not divided. It is assumed that the two original nuclei did not divide at exactly the same time. 7, a hyphal tip in which a single nucleus only was visible. In 8 the upper pair of nuclei probably have resulted from the division of a single nucleus, while the lower pair are just separating. A short time earlier this hyphal tip probably would have appeared to be 3-nucleate. 13. A promycelium on which three dikaryotic hyphae have developed; fusion tube between two of the sporidia still visible. 14-15. Tips of very long hyphae such as those shown in 13, with the nuclei distinctly arranged in two pairs. 16. H-shape fusion between hyphal tips. Unfortunately, the nuclei were not visible. 17. Fusion between the tip cells of two long hyphae that appeared to be uninucleate. The nucleus from the upper one is passing into the lower one which already is sending out a hypha. These hyphae could be traced through a large number of empty cells to different clumps of spores. 18. The spore has sent out a long germ tube, some of the empty cells of which are not shown. Two of the cells have become binucleate and are sending out hyphae, while the tip cell has turned backward and fused with the first living uninucleate cell. The nucleus from the latter appears about to pass through the fusion tube into the tip cell, which apparently has begun to elongate into a hypha.

mation of 4 or more nuclei, 4 and 6 being the most common. Normally, one nucleus passes into each sporidium, often after the sporidia have attained almost their maximum size. Regardless of the number of sporidia, each contains a single nucleus prior to sporidial fusions, with the exceptions noted below. A nucleus may or may not be visible in the promycelium after each sporidium has received one (Plate I, 13 to 18).

As soon as the sporidia are fully formed they tend to fuse (Plate I, 19 to 23, and text Fig. 1). There seems to be no fixed method for fusion. H-shape fusions, such as those in *Tilletia* spp., occur frequently (Plate I, 10, 11, 19, and 22; Plate II, 1 and 3); basal fusions, as described by Kniep for *Urocystis anemones*, are common (Plate I, 7 and 20, and text Fig. 1, A and B); and tip fusions occur frequently (Plate I, 20, 21, and 23, and text Fig. 1, C and D).

Not only is the method of fusion variable, but the nuclear behavior, also, varies somewhat. The simplest case is shown in plate I, 19. There were only 2 sporidia on the promycelium; they have become united by a fusion tube; and the nucleus from the sporidium on the right has passed into that on the left, which has begun to elongate into a hypha. The sporidium on the right already is partially vacuolate. In plate I, 23, two sporidia on the promycelium from the upper cell of the spore have fused at their tips; and the nucleus from the one on the right is passing over to the one on the left, which already has begun to send out a hypha. The sequence of events is slightly more complex in 20, 21, and 22 of plate I. In 22 there is a fusion tube between sporidia *b* and *c*; the nucleus from *b* is just passing through the fusion tube into *c*, in which a septum has been formed just below the fusion tube. The lower cell of *c*, which appears to contain a nucleus, is about to fuse with the third sporidium, *a*. It is not entirely clear whether the nucleus of sporidium *c* divided, as one could not be certain that the dark-staining body near the base is a nucleus. If it is not, presumably the nucleus in the promycelium would move up. In 21 there is a similar situation. Sporidia *a* and *b* have fused near their tips, and *b* already is elongating into a hypha. A septum has been formed just below the point of fusion in *b*, and the lower cell probably will fuse with sporidium *c*, which appears to be sending out a protuberance at the base. Since young, non-fused sporidia are almost universally uninucleate, it is probable that sporidium *b* originally contained a single nucleus that subsequently divided, giving rise to 2 sister nuclei, although it is possible, of course, that 2 nuclei migrated directly from the promycelium into the sporidium. In 20, sporidia *a* and *b* have fused near the base by means of a broad fusion tube, while the tips of *c* and *d* have fused and a hypha is growing out from *d*. Cross walls have formed in *c* and *b* and the upper cell of the latter and the basal cell of the former appear to be about to fuse, as there is a slight protuber-



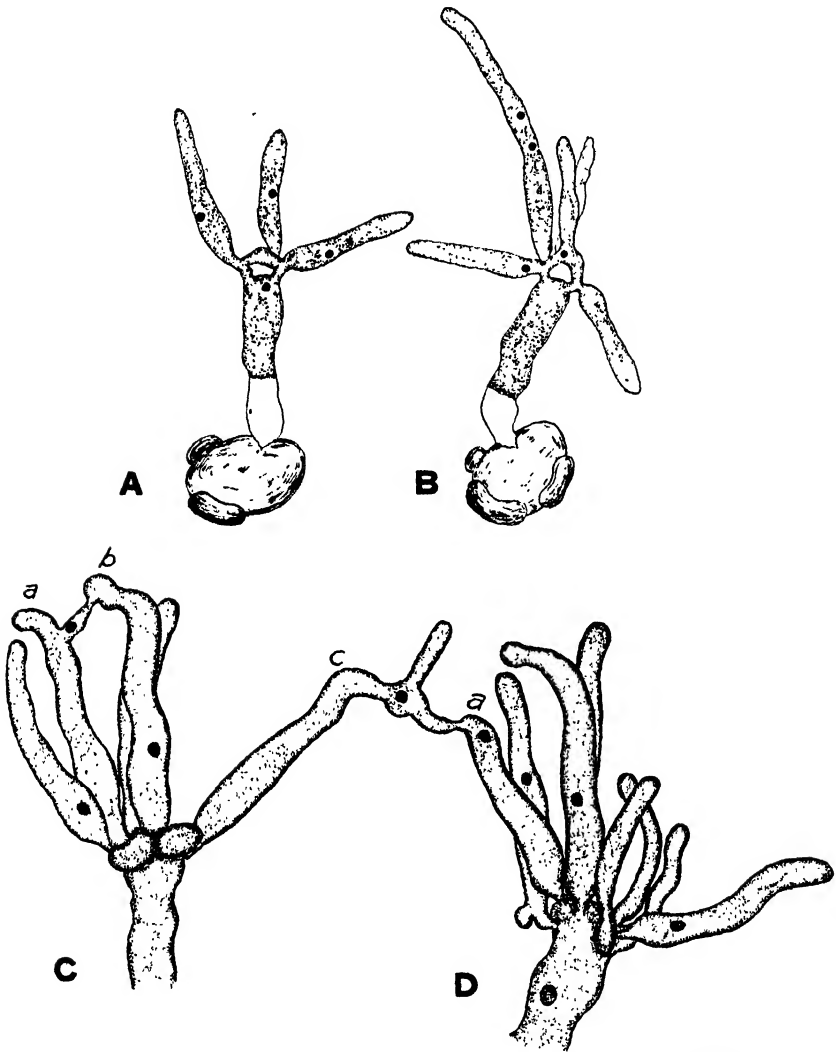


FIG. 1. A and B. Typical basal fusions between sporidia. A. The fusion tube between two sporidia is complete, but the nuclei have not yet paired. B. Two sporidia connected by a fusion tube that is sending out a hypha into which the two nuclei apparently are passing. The other two sporidia evidently have fused also; one is binucleate and is elongating into a hypha. The connection could not be seen, however. C and D. Two promycelia showing sporidial fusions. On promycelium C sporidia *a* and *b* have fused; the nucleus from *a* already in the fusion tube and passing over toward *b*. There are basal fusions between some of the sporidia also, but the exact connection could not be made out. Sporidium *c* of promycelium C has fused with sporidium *a* of promycelium D by means of a long fusion tube, which is already sending out a hypha. The nucleus from *c* is about to enter this hypha, while that from *a* is about to pass into the fusion tube. There is a large nucleus in promycelium D; note nucleolus.

ance on the *c* and a pronounced one on the *b*. For the sake of clearness, these 2 sporidia are shown farther apart than they originally were. During the extremely hot weather of August the balsam on the slide became soft, and by pressing the cover slip gently the sporidia could be made to move into almost any desired position. They were drawn in the most favorable position for showing fusions; hence the likelihood of fusion between *c* and *b* does not seem so strong from a study of the drawing as it did from a study of the material itself. Sporidia *a* and *c* would seem to be of one sex and *b* and *d* of another.

Sporidia from different promycelia may fuse also, as shown in text figure 1. A hypha already has begun to grow from the swollen portion of the rather long fusion tube between sporidium *c* of promycelium C and sporidium *a* of promycelium D. Sporidia *a* and *b* of promycelium C also have fused, and it is likely that there are basal fusions between some of the other sporidia, also, but it was not possible to distinguish them clearly.

Very soon after fusion, even before the nuclei have become closely paired, one of the fused sporidia, or the fusion tube itself, sends out a hypha, while the other sporidium becomes vacuolate and often collapses, as is true also of the nonfused sporidia. Early stages in the development of the hyphae are shown in the figures in plate I, already alluded to, while somewhat later stages are shown in plate II, 2, 3, 4, 5, and 13.

From 1 to 3 hyphae develop on a single promycelium. Usually they are binucleate in the early stages, but later there often are 2 pairs of nuclei. In a few hyphae there appear to be 3 nuclei, and very rarely there seems to be only one.

Nuclear counts were made in 100 hyphae on several slides on which various stages of germination were represented. Similar hyphae are shown in plate II, 6 to 15. In one hypha there was one nucleus; in 47 there were 2; in 4 there were 3; and in 48 there were 4.

Ninety-five per cent of the hyphae, therefore, had either 2 or 4 nuclei, often arranged distinctly in pairs, as shown in plate II, 14 and 15. Those having 3 nuclei may have been in the dikaryophase, also, but were temporarily 3-nucleate because the 2 original nuclei did not divide at exactly the same time. In plate II, 6, for example, the lower nucleus of the pair has just divided, while the upper one has not yet divided; and in plate II, 8, the upper nucleus apparently has divided and the daughter nuclei are a short distance apart, while the lower nucleus is just completing division, the daughter nuclei still lying close together. A few minutes earlier this hypha probably would have had 3 nuclei. It is possible, too, that the fourth nucleus sometimes is not visible, as it is only in the best-stained slides that all nuclei can be seen clearly. Because of these facts and the fact that the young hyphae are almost universally binucleate or 4-nucleate (Plate II,

2 to 5), it seems most probable that the older hyphae in which 3 nuclei were counted either were only temporarily in this condition, owing to a slight difference in the time of division of the 2 original nuclei, or that the fourth nucleus was not visible, either because of imperfect staining or possibly because of the position of 2 nuclei in approximately the same vertical plane, one superimposed on the other. If this is true, the percentage of hyphae in the dikaryophase was 99 per cent and only 1 per cent appeared to have a single nucleus. Here again, however, the second nucleus may not have been visible for the reasons mentioned above or the hypha may have been binucleate at first and may later have become uninucleate.

That a few hyphae actually are uninucleate is suggested by such conditions as shown in plate II, 7 and 17. The hyphal tip shown in 7 was well stained and the only visible nucleus was very distinct. The 2 hyphae shown in 17 were uninucleate. They could be traced back, along many empty cells, to different clumps of spores, and the 2 cells at the tips have fused; the nucleus from the upper hyphae is passing into the lower one, which is sending a hypha out toward the right. It seems probable, therefore, that older hyphae may occasionally be uninucleate and may fuse with each other to produce binucleate hyphae.

Apparently the dikaryophase sometimes is initiated without previous fusion. (See Plate I, 24 and 25.) In both of these cases there was no evidence whatever of fusions; nevertheless, in 24 the sporidium on the left has sent out a hypha that contains 4 nuclei, which apparently came directly from the promycelium. The other 2 sporidia contain no visible nuclei and are degenerating. In 25 the 2 large sporidia are binucleate and there is no indication whatever of fusions, as was agreed by at least 6 competent observers. In plate II, 18, is shown a promycelium that grew directly into a long germ tube, 2 of the cells of which are binucleate and are sending out hyphae, while the uninucleate end cell has curved backward and is fusing with another uninucleate cell.

It is clear from the above that the dikaryophase may originate in several different ways. The normal condition is for the uninucleate sporidia of the same promycelium to fuse, either at the base, or by means of H-shape fusions, or at the tips; but sporidia of different promycelia also may fuse. Occasionally, rather long, uninucleate hyphae apparently develop, and these may then fuse and send out a typical dikaryotic hypha. In one case, at least, all the nuclei from the promycelium apparently passed directly into one sporidium, which then developed further, while the others disintegrated. In still another case 2 sporidia on a promycelium each contained 2 nuclei, and, as there were only 2 sporidia on this promycelium and they had not fused, it is presumed that the nuclei paired before passing into the sporidia, although it is possible that each sporidium originally contained one

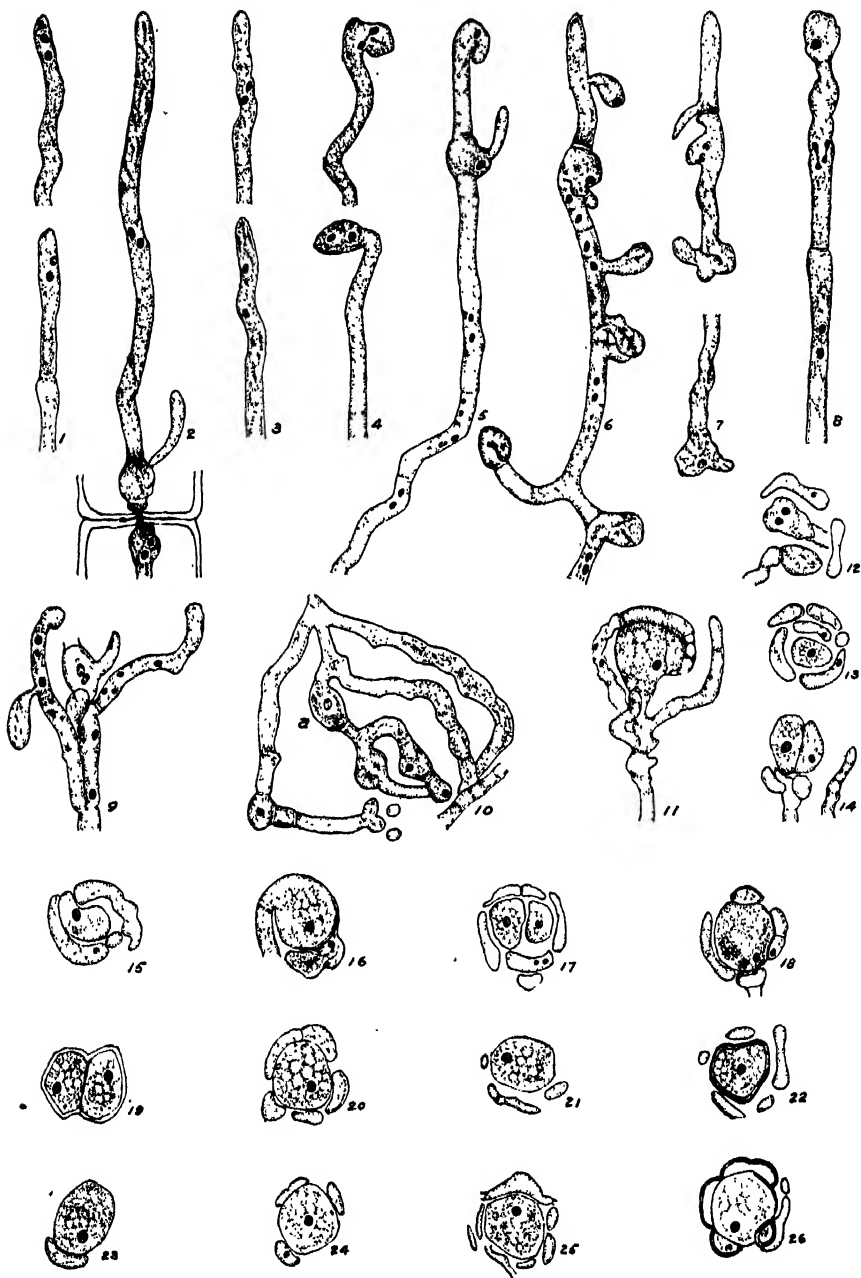


PLATE III

nucleus, which divided in the sporidium. One promycelium was observed that grew out directly into a long germ tube in which 3 cells became binucleate, one by fusion with another cell of the same tube.

Apparently, therefore, several haploid nuclei ordinarily result from the division of a single diploid nucleus in the young promycelium. These haploid nuclei normally pair after fusion of sporidia, and exceptionally, perhaps, after fusion between uninucleate hyphae. It seems significant that the characteristic dikaryotic hyphae begin to develop very soon after plasmogamy. The presence of 2 nuclei in a protoplast, even when they are still a considerable distance apart, imparts to it an ability that the haplophase does not seem to possess.

It would seem that infection of the host must result from invasion by hyphae of the dikaryophase, as only such hyphae seem to be capable of appreciable development. As far as our observations go, 48 hours after the spores began to germinate there were virtually no hyphae except those of the dikaryophase. The writers are quite aware, however, that there is considerable variability in the development of smut fungi and would not

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#### PLATE III

The mycelium in the host and spore formation. 1-3. Vegetative hyphae in the rye plant, showing the binucleate condition. In 2 the mycelium is intracellular and has forced its way through the transverse wall between two parenchyma cells. Both the lower cell and the upper cell are clearly binucleate. 4. Swollen tips of hyphae that are still binucleate. 5. A hypha in which the basal cells are binucleate, and two large uninucleate cells apparently rounding up into spore mother cells. 6. A bit of mycelium in which there are four successive binucleate cells and two uninucleate ones, the lower of which evidently is a spore mother cell. 7. Above, a cell beginning to swell up into what may become a spore mother cell; below, a tip cell that has become uninucleate. 8. The basal cell is distinctly binucleate. The cell immediately above it contains two nuclei that seem to be in process of division, while the upper cell is distinctly uninucleate and is probably a spore mother cell. The hypha may have continued beyond the upper cell, but, because of its gnarled condition, could not be traced farther. 9. A spore mother cell in which the nuclei are about to fuse; hyphae, which presumably will form the sterile cells, growing up around it. 10. Sporulating mycelium. Cell *a* appears definitely to be a spore mother cell. It is probable that some of the surrounding hyphae may become attached as sterile cells. 11. A later stage in spore formation. Apparently, the next to the last cell in the hypha is rounding up into a spore mother cell, while the tip cell has curved downward and may also become a fertile cell. The hyphae from below are curving around these cells, some of them already having become appressed. A spore with two fertile cells probably will result. 12-13. Young spore mother cells with surrounding hyphae. 14. The formation of a 2-celled spore. The hyphae at the base, partly cut off, apparently were growing around to form sterile cells. 15-26. Various stages in the development of spores. It will be noted that each young spore is uninucleate, although there is a dark staining body in 18 that may possibly be a second nucleus. In 17 two nuclei are still visible in one of the sterile cells. In most of the others single nuclei or none are visible.

be surprised if there is still more variability in *Urocystis occulta* than that they observed.

*Mycelium and Spore Formation in the Host.* Material from smutted plants growing in the greenhouse was killed in Carnoy's solution or in Flemming's weaker solution, embedded, and sectioned in the usual manner. Both cross and longitudinal sections were made through sori in various stages of development. Sections from 5 to 10  $\mu$  in thickness were stained in various ways, mostly with the triple stain or Heidenhain's iron-alum haematoxylin. The latter proved by far the most satisfactory, especially with a counter stain of Orange G or eosin.

It is rather difficult to find many vegetative hyphae in which the nuclei can be seen in a considerable number of consecutive cells, because the hyphae are likely to be somewhat gnarled and sporulation may begin soon after a sorus primordium is formed.

Clearly, however, the cells of the vegetative hyphae are usually binucleate, as shown in plate III, 1 to 6. In the early stages of spore formation some of the cells enlarge considerably. They may still be binucleate, even after having attained considerable size (Plate III, 9, 12), or the nuclei may already have fused when the spore mother cells are not very much larger than the other cells (Plate III, 5, 6, 10, 13). By the time cells have begun to look like spores, however, they are virtually universally uninucleate. The nucleus is rather large and a nucleolus is usually clearly visible. By this time, the other cells of the hyphae, except those that develop into sterile cells, are in process of disintegration. The sterile cells are formed from hyphal branches that grow up around the spore mother cell from neighboring cells. The cells of these branches appear to remain binucleate; they become closely appressed to the enlarging spore mother cells, attach themselves to them, and assume various shapes and sizes; their protoplasm and nuclei disappear; and the walls thicken and become dark, as do those of the spores, and the spore is complete (Plate III, 15 to 26).

#### DISCUSSION

The cytological phenomena in *Urocystis occulta* are similar to those described by Kniep for *U. anemones* and Rawitscher for *U. violae*, except that there is no rigidly fixed method for the initiation of the dikaryophase. Whenever 2 haploid cells containing nuclei of opposite sex are close together they fuse and the nuclei become paired; and the exact place or method seems of secondary importance. By far the most common method is through some kind of fusion between sporidia, but it may occasionally result from the direct passage of 2 or more nuclei from the promycelium into a sporidium, or, very rarely, from the fusion of uninucleate cells in fairly long hyphae.\* The various methods of fusion indicate clearly that

in this species, as in the other smut fungi, sex is reduced to very simple terms. The gametes are nothing more than haploid nuclei and there certainly are no special gametangia. Consequently, the concept of sex in *U. occulta* must be pushed back to the bare essentials of the phenomenon. The sexual process is the association and union of two haploid nuclei containing different factors for sex, with the resulting formation of a zygote. The really important fact is that there are different factors in different haploid nuclei. If, in addition, in some fungi, there are different factors for growth and for the formation of morphologically different gametes and gametangia, the sex differences become obvious and maleness or femaleness can be distinguished on the basis of characteristic structures. But this additional evidence of sex is merely an elaboration. As concerns the implications of the sexual process, nothing essential has been added by the formation of sex organs, except structures that enable a recognition of sex differences by morphology instead of behavior. The writers are of the opinion that the phenomena in *U. occulta* support this view. Haploid nuclei become associated whenever opportunity offers, but how and where seem to be of relatively little importance, as is shown by the variability of the method of fusion between cells containing them.

While the haplophase of such species as *Ustilago zaeae*, *Sphacelotheca sorghi*, and others may persist for a long time, saprophytically, by the budding of sporidia, the haplophase in *Urocystis occulta* seems to be restricted principally to the sporidia and the promycelium. Normally, therefore, it would last only a few days in nature, and almost the entire active life of the organism would be passed in the dikaryophase, as the diplophase is restricted to the spore mother cells and chlamydospores.

Those who are still loath to interpret sporidial fusions in the smut fungi as part of a sexual process may be skeptical about the sexual nature of the nuclear phenomena in *Urocystis occulta*. It must be admitted, of course, that there is only circumstantial evidence that the single large nucleus in the very young promycelium is diploid, that one of the several successive divisions is a reduction division, and that the nuclei in the sporidia are haploid. However, the fact that there clearly is an affinity between certain sporidia, resulting in fusions of various kinds, seems strong evidence that their nuclei have different factors for sex. Furthermore, one of the fused sporidia usually sends out a hypha almost immediately after the fusion tube becomes continuous, thus uniting the 2 protoplasts. The combined effect of the 2 nuclei in stimulating vigorous growth, even when still separated by a considerable distance, is noteworthy. Binucleate cells have an ability that uninucleate cells apparently do not have. Finally, the rather distinct pairing of the nuclei in 4-nucleate hyphae seems significant, and, certainly, the fusion of paired nuclei at the time chlamydospores are formed can scarcely be anything but the final act in the sexual processes.

As concerns the pathogenicity of the organism, the principal interest in the sexual process is the degree to which it permits recombination of factors for pathogenicity. This could best be studied by cutting off individual sporidia, growing them in culture, and then mating them in various combinations. However, the opportunity for variation resulting from hybridization in *Urocystis occulta*, in which the sporidia do not seem to become detached from the promycelium and apparently do not bud to any extent, seems far more limited than in *Ustilago zeae* and similar smuts, which produce countless sporidia that may multiply saprophytically and be disseminated widely by the wind. Naturally, the number of genetic factors for pathogenicity will determine the number of possible combinations, but in smuts of the *U. zeae* type there is a condition roughly analogous to cross pollination in higher plants. In *Urocystis occulta*, on the other hand, it is more nearly analogous to self pollination, because, ordinarily, only the haploid derivatives of a single diploid nucleus would become paired, unless further nuclear associations occur in the host plant, where the mycelium is already in the dikaryophase. There is, of course, the possibility of "wider crossing" when sporidia of different promycelia fuse. It is possible, also, that, under certain conditions, haploid propagative bodies may multiply to a limited extent, although the writers have thus far obtained no evidence that this may be true.

Theoretically, therefore, there should be fewer parasitic races in *Urocystis occulta* than in *Ustilago zeae*. Whether this is true has not been determined definitely. The writers have inoculated several varieties of rye with different collections of rye smut but found no clear-cut indications of significant differences in pathogenicity. The tests, however, have not been extensive enough to justify definite conclusions.

#### SUMMARY

The spores of *Urocystis occulta* germinate readily in about 48 hours, producing typically a promycelium of variable length on which are formed from 2 to 6 sporidia that apparently always remain attached to it.

There is a single nucleus in the young promycelium, but several nuclei are formed as a result of successive divisions.

Normally, one nucleus passes into each sporidium, certain sporidia on the same promycelium or different ones then fuse in various ways, a binucleate hypha grows out from one of the fused sporidia or from the fusion tube, and the sporidium from which the nucleus has migrated degenerates and often collapses, as do the nonfused sporidia. This is the usual method by which the dikaryophase is initiated.

Rarely, cells of fairly long hyphae apparently are uninucleate. They may then fuse with other uninucleate cells.



Occasionally, two or more nuclei pass directly from the promycelium into a sporidium, and the dikaryophase begins without previous sporidial fusions.

The host plant probably is infected by hyphae of the dikaryophase. These hyphae may have many cells, but the protoplasm usually is aggregated in the tip cell. Several days after spores have germinated most of the hyphae contain 2 or 4 nuclei, arranged in 2 pairs. Some hyphae seem to contain 3 nuclei, or, rarely, a single nucleus. The 3-nucleate condition probably is often temporary, due to a slight difference in the time of division of the 2 original nuclei or in the separation of the daughter nuclei.

The hyphal cells in the host plant are predominantly dikaryotic until spore formation begins. Then the nuclei in some of the cells fuse, and these spore-mother cells enlarge rapidly at the expense of the others. In the meantime, hyphal branches, often dikaryotic, grow up around the spore-mother cells, appress and attach themselves to them, and lose their protoplasm, the walls of the fertile and sterile cells thicken, and the spore is formed.

There would seem to be less opportunity for variation resulting from hybridization in *Urocystis occulta* than in many other smut fungi.

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# A LEAF, STEM, AND POD SPOT OF PEA CAUSED BY A SPECIES OF CLADOSPORIUM<sup>1</sup>

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## INTRODUCTION

In the fall of 1932 growers and packers of market peas in certain coastal areas of California directed attention to an apparently undescribed pod spot of peas, *Pisum sativum* L. Field examinations revealed the occurrence of irregular to roughly circular necrotic spots on the foliage, resulting in distortion, and cankers on the stems as well as dark brown to black scabs of irregular shape, which seriously marred the pods. Young, rapidly growing tissue was most affected. Microscopic examination revealed the consistent association of a *Cladosporium* with the spots. Culture methods readily effected recovery of the causal fungus, which, successfully used in inoculation trials, is herein described as *Cladosporium pisicolum*, n. sp. Distinctly different from other pea diseases, this is typical of other *Cladosporium* diseases, notably the *Cladosporium* spot of cowpea, caused by *Cladosporium vignae* Gardner (5).

Occasional references may be found in the literature associating species of *Cladosporium* with diseased peas. In 1891 Cugini and Macchiati (1, p. 104) described a pea disease in Italy, which they attributed to *Cladosporium pisi*, n. sp. Affected pods dried prematurely and bore scattered, elevated pustules upon which the fungus sporulated. Curzi and Barbaini (2) in 1926 refuted the earlier work of their countrymen by demonstrating that the pod disease was not of parasitic origin but the result of intumescences incited by such factors as temperature, light, and humidity, and that *C. pisi*, which they conclude is *C. herbarum* Link, follows the intumescences as a secondary factor. The descriptions and illustrations of both Cugini and Macchiati and Curzi and Barbaini bear out the conclusions of the latter and clearly set apart the Italian disease from that to be presently described. *C. myriosporum* E. and D., with minutely echinulate conidia, was reported in pea pods in Canada (4) in 1897. Dowson (3) in England described a new disease of the sweet pea in 1924, caused by *C. album*, n. sp., a fungus morphologically identical with *C. herbarum* but white, giving a mildewed appearance to the leaves. Dowson obtained no infection of *Pisum*. In the

<sup>1</sup> Contribution from the Division of Plant Pathology, University of California, Berkeley.

<sup>2</sup> The writer gratefully acknowledges the counsel given by Professor Ralph E. Smith and Dr. Max W. Gardner.

United States reference was made to a "black spot of peas caused by *Cladosporium* sp. on the seeds" in Washington by Heald (6, p. 49) in 1930. A preliminary report of a leaf, stem, and pod disease of market peas in California was later made by the writer.<sup>3</sup>

#### DISTRIBUTION

The writer has observed the disease only in California, although it is not unreasonable to expect its occurrence in other pea areas along the Pacific Coast. It may well be that the black-spot disease referred to by Heald is of the same cause. Of the 4 major market pea areas in California, which include Alameda, Monterey, San Luis Obispo, and Imperial Counties, all but the last have been visited the past year. Among these the disease was encountered in most severe form in Monterey County, especially in the Castroville and Salinas district situated along the ocean shore.

#### ECONOMIC IMPORTANCE

The blighting of young seedlings, interference with proper vegetative development of the vine, and scabbing of the pods are phases of the disease contributing to economic loss. Observations have shown that young seedlings may be killed when badly infected and others so deformed as to be permanently retarded. During certain periods when environmental factors favor the disease, appreciable quantities of blemished pods must be culled out at the packing sheds, resulting in a reduction of 5 to 10 per cent or more of the picked crop. Blighting of the blossoms and very young pods accounts for further losses in yield.

#### DESCRIPTION OF SYMPTOMS

Foliage symptoms are manifest as roughly circular or irregular discolored spots, which, in early stages and under humid conditions, become covered with a velvety, smoke-colored sporulation. Later, they appear as necrotic tan or brown spots, involving the thickness of the leaf or stipule and sharply delimited generally by a thin line of dark brown to purple (Fig. 1, B). Spots may occur any place within the leaf edge, or marginally, resulting in rapid necrosis of the affected area, which varies from 1 to 8 millimeters in diameter. Where marginal infection takes place marked distortion accompanies continued growth of the leaf, and when such infection is general the cupped or curled, twisted appearance of the foliage (Fig. 2, A, B) becomes recognizable some distance away. Tearing and

<sup>3</sup> Wood, Jessie I., Neil E. Stevens, and Paul R. Miller. Diseases of plants in the United States in 1932. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Reporter Sup. 85. 1933. (p. 53-54)



FIG. 1. Symptoms of *Cladosporium* spot on naturally infected pea. A. Sunken lesions on stems, showing the small lens-shape, crater-like infections as well as long narrow cankers parallel to the vertical axis of the plant. The coalescence of lesions results in irregularly shaped cankers. B. Spots upon the stipules and leaflets occur marginally or interiorly as roughly circular necrotic areas bounded by narrow dark borders. Tearing of the parchment-like lesions gives a tattered, frayed appearance to foliage bearing older infections. Blossom spots do not show well in the photograph, but heavy infection of the attached pod is apparent. These lesions are numerous and mostly small and dark brown, and are sporulating in some cases. C. An advanced pod infection. The black, irregular scabs become sunken in the center with a raised, sharply defined black border. Sporulation on the scabs is abundant with high humidity.

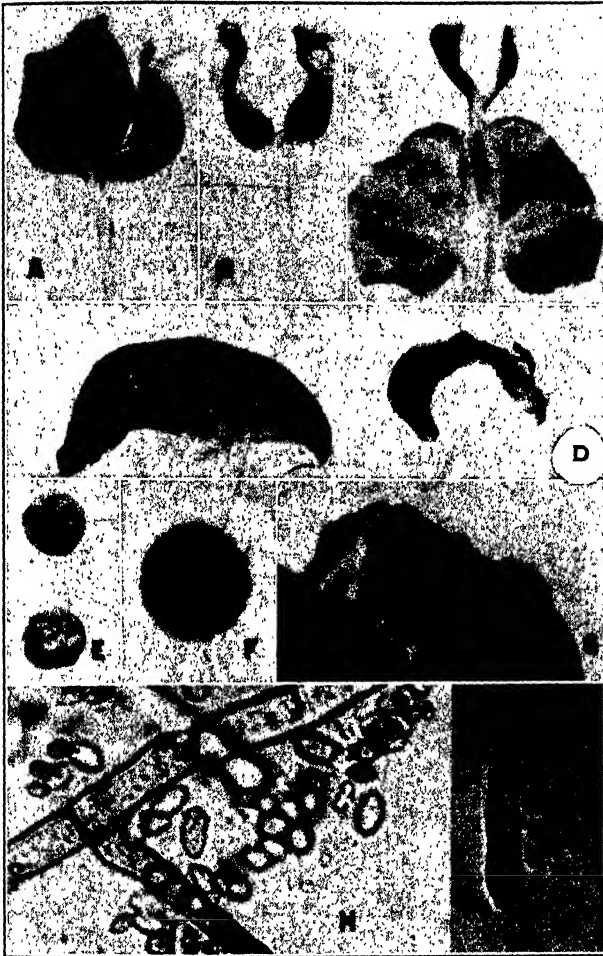


FIG. 2. A-B. Leaves of young pea plants 6 days after atomizing with a spore suspension of *Cladosporium pisicolum*. Sporulation could be observed on the lesions of each leaflet. Note especially the rolled, misshapened appearance, which becomes exaggerated as the unaffected portions of the leaflets continue their development. C. The first stipules and leaflets of a pea seedling grown in infested soil, showing primary infections incurred during emergence. Also typical of primary infections from diseased seed. D. Secondary infections on pea pods from outdoor plants artificially inoculated in the seedling stage 2 months earlier. Observe the severe distortion of the pod on the right. E. Seed naturally infected through pod invasion as the result of secondary spread on plants artificially inoculated 2 months earlier. F. Colony of the pea *Cladosporium* from a dilution plate on potato-dextrose agar held 8 days at room temperature. G. Sporulation upon a pea leaflet 6 days after inoculation in a moist chamber.  $\times 4$ . H. Photomicrograph of mycelium and spores, showing the predominant small conidia and one large 2-cell spore.  $\times 900$

fraying of the parchment-like areas of old spots, particularly when marginal, give a tattered appearance to leaves bearing many lesions. These may turn yellow and wither prematurely.

On growing stems, petioles, peduncles, and pedicels, lesions occur first as narrow dark brown or black streaks parallel to the long axis of the affected organ. With continued growth of the part this linear lesion results in a fissure or crack in the tissue. Frequently, the lesions develop into cankers 1 to 4 mm. wide and 1 cm. or more in length, the smaller ones becoming lens-shape (Fig. 1, A, B). With coalescence the cankers extend in irregular patterns over considerable areas and even encircle the affected organ. The lesions are at first slightly sunken. As they enlarge under favorable circumstances, accompanied by continued growth of the part, the lesions deepen and become somewhat crater-like as the result of a raised, ridge-like border. Under moist conditions the crater may be filled with sporulation of the fungus. One sometimes finds severely cankered stems where up to 50 per cent of the surface of an internode may be deeply etched with lesions. The base of the internode where moisture collects under the protecting influence of the stipules is often most severely infected. The well-defined lesions are light to dark brown in the center with a narrow, dark brown to purplish black border. Pedicel lesions sometimes extend over into pod tissue, initiating infection of the fruit, and calyces are frequently infected.

Pod infection often begins beneath dead floral parts, which at first encase and, for a time, adhere to the young pod, upon which the fungus may sporulate. The dark brown or black, very irregular, definitely margined scabs on the pods constitute one of the conspicuous phases of the disease. The scabs, sunken or raised, occurring numerous, as brownish or blackish specks (Fig. 1, B) or sparsely, as large ugly blemishes (Fig. 1, C), give a roughened feel and unsightly appearance to the fruit. Usually measuring 1 to 9 mm. or more in length, the lesions may run together to cover portions of the pod, and, where young ovaries are concerned, cause distortion of the developing structure (Fig. 2, D). Several instances have been encountered where the scab lesions on green pods penetrated the pod wall and discolored 1 or 2 seeds, and more rarely sporulation within the pod cavity has been found, at once suggesting that the fungus may be carried with the seed. Seed infections appear as brown to black spots, which may be scattered over the seed surface as tiny specks or occur as lesions several millimeters in diameter (Fig. 2, E). Small blisters or elevated scabs are produced when immature seed becomes infected, and upon these spots the fungus sporulates (Fig. 3, F). Old seed infections appear as black, roughly circular spots with well-defined borders and may be sunken or crater-like.

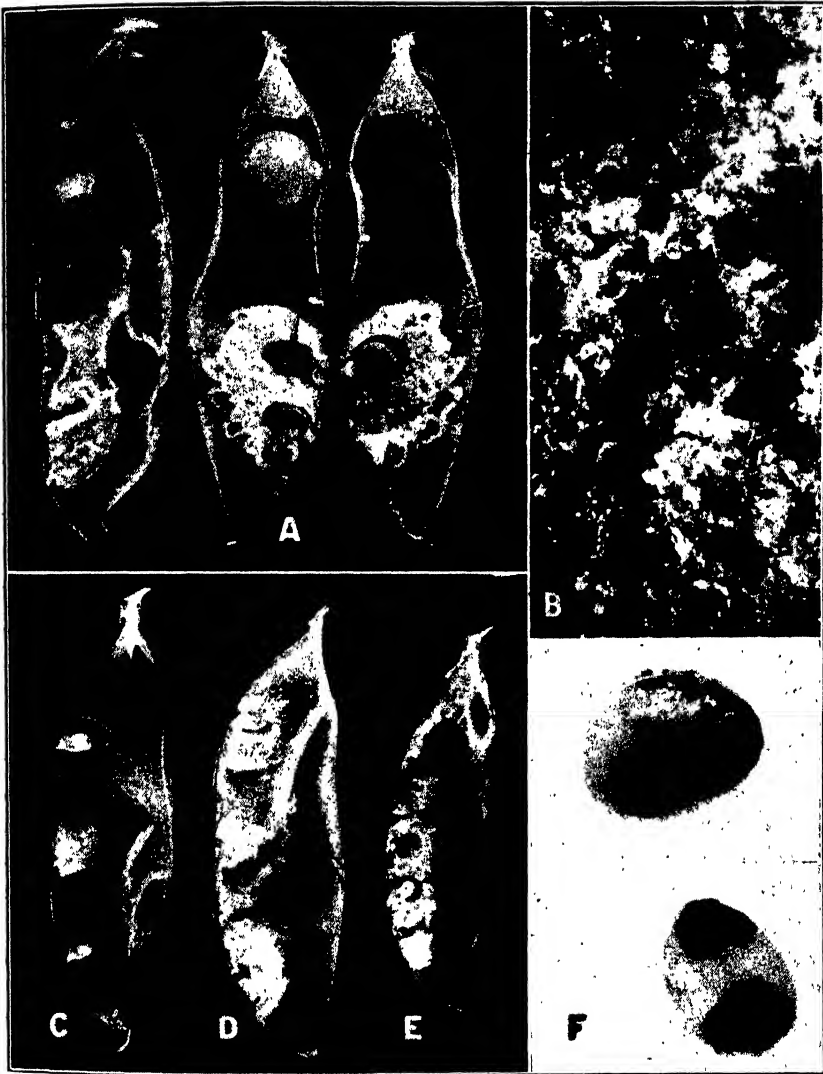


FIG. 3. Results of hypodermic treatments upon attached, growing pea pods. A. Internal *Cladosporium* infection 12 days after a hypodermic injection of a spore suspension into the pod cavity. Observe the white, felty proliferation containing spots of infection and sporulating lesions upon the seed. B. Proliferated cells of the inner pod membrane, as seen in A.  $\times 22$ . C-E. Comparison of effects of different hypodermic treatments. C. Air only was forced into the pod. A small area of proliferation has developed about the needle wound. D. Result of injection of a spore suspension as in A. E. Water only was introduced into the pod cavity. The proliferation is much milder and less dense and does not contain the spots of *Cladosporium* infection obtained in A and D. F. Blistered, lesioned seed from pods treated as in A.  $\times 5$ .

## ETIOLOGY

The fungus, a *Cladosporium*, was first isolated from scabby pea pods taken from the cull bin of a packing shed near Castroville, in October, 1932. Marginal tissue of each scab, when cultured, yielded an essentially pure culture of the organism. Several of the isolates were selected to inoculate healthy Hundredfold pea plants a month old, growing out-of-doors. The method of inoculation consisted in spraying the vines with a water suspension of fungus spores, by means of a DeVilbiss atomizer. Inoculation was made in the evening, following which bell jars were left over the plants for 14 to 24 hours. Leaf spots appeared upon the inoculated foliage in less than a week, and from these young lesions the *Cladosporium* was recovered in pure culture and used to similarly inoculate plants of the Laxton's Progress, American Wonder and Hundredfold varieties in the greenhouse. Infection was obtained on each variety in the form of foliage spots, while control plants, atomized with water alone, remained healthy. Inasmuch as 4- and 5-week-old vines had been used in these tests, the next inoculation trial was made on plants only 12 days removed from the date of sowing, averaging at this time 2 inches in height, with the first leaves unfolding. Gardner (5) has shown, in this regard, that cowpeas are most susceptible to *Cladosporium vignae* at the time young leaves are just unfolding. From 12 to 14 young pea seedlings, representing each of several varieties, were placed in a large cloth insect cage, in place of bell jars because of greater convenience and thoroughly atomized with a spore suspension derived from a monosporidial culture of the fungus. The experiment was conducted in a greenhouse running at 60° to 70° F. with relatively high humidity; but, as an extra precaution, the plants were given 2 additional sprayings with water only, within the 24 hours following inoculation. Six days later an extensive infection was evident, examples of which are illustrated in figure 2, A, B. Of the 6 seed lots used, Hundredfold, Alderman, Laxton's Progress, Wisconsin Early Sweet, Laxtonian, and Stratagem, all developed infection, ranging from 40 to 100 per cent within the 6 days following inoculation. The degree of infection was moderate to severe. Subsequent controlled experiments, using plants coming into pod, have also proved successful in the artificial inoculation of the fruit by means of an atomized spore suspension, with the production of typical pod scabs.

These first experiments demonstrated the parasitic nature of the *Cladosporium* in question. Later, pure cultures from monoconidial lines were used, and these also were productive of a rapid and severe disease of all aboveground parts of growing pea plants, the first symptoms appearing in about 3 days, whereas like plants, similarly treated, with the exception that water instead of a spore suspension was sprayed on them, developed into



healthy, normal vines. Attempts to infect cowpea, to which reference will be made later, proved unsuccessful.

#### MORPHOLOGY AND TAXONOMY

Colonies of the fungus obtained upon dilution plates of potato-dextrose agar (Fig. 2, F) become visible within 2 days, when held at room temperatures. At this age the colonies average 1 mm. in diameter and are already bearing spores, the dusty layer of which gives a vetiver green<sup>4</sup> color to the colonies for the first 2 to 5 days. In less than a week the color becomes modified to deep olive and finally turns olivaceous black with age. Viewed from the underside of the plate the colony assumes a dull greenish black within a few days, which does not appreciably change except to darken somewhat. On potato-dextrose-agar slants the colony, instead of remaining flat on the agar surface, becomes convoluted and wrinkled, and this is also true of plate cultures held at the maximum temperature limits for the fungus. Small dots of grayish white mycelium frequently appear as secondary growth phases on old cultures of the fungus but remain much limited in extent.

The mycelium, which may be relatively hyaline in the interior of infected tissue, where it rapidly ramifies in a much-branched, twisted, septate condition, appears greenish brown or smoke colored, curved but not twisted, and is thicker walled, when on the surface. From this stromatoid mycelium arise the conidiophores, which, with their clusters of conidia, occur abundantly enough to produce a velvety appearance of the infected area (Fig. 2, G). In artificial culture on potato-dextrose agar all the mycelium is dark-colored, especially that part of the colony that is submerged. A dense stand of conidiophores results in a continuous layer of conidia over the surface.

The upright, sometimes slightly crooked, conidiophores are unbranched, dark brown, one to several times septate, and frequently measure 100  $\mu$  and more in length. A slight jog or crook may be observed in the region of a septum, marking the previous attachment at that point of a conidial chain. Occasionally, with special precautions in mounting, one may observe 1 to 3 spore clusters, laterally intact at these points, in addition to the terminal one.

The conidia are borne acrogenously in clusters of multibranched spore chains. The basal cells supporting the conidial chains are 2 to 4 times longer than the general run of conidia, cylindrical to cigar-shape, and arise from the sporophore singly or in groups of 2 to 4 but predominantly in groups of 3. On these basal spores are cut off the succeeding conidia,

<sup>4</sup> Ridgway, R. Color standards and color nomenclature. 43 pp.; 53 col. pls. Washington, D. C. 1912.

which diminish in size towards the tips of the chains. The spore chains may consist of 1 to 9 or more conidia and, in addition, may give rise laterally to several branched chains each of which again branches, resulting in a many-chain cluster.

The conidia are light brown, ellipsoid with somewhat pointed ends, or ovoid, and are predominantly continuous, although infrequently one-septate. The larger spores, also brown, are cigar-shape with the base sometimes thicker than the apical portion, continuous or one-septate, occasionally two-septate, and tend to be slightly pedicellate. The smaller spores vary in length from 5.2 to 12.5  $\mu$  and 3.7 to 5.5  $\mu$  in width, while the larger spores range in length from 12.5 to 23.7 and in width from 4.0 to 5.5  $\mu$ . These measurements, based upon the sporulation of week-old lesions, are essentially the same for agar cultures, although the average dimensions of spores produced on lesions tend to be slightly higher.

In examinations of the fungus the writer soon appreciated the close resemblance it bore to the genus *Hormodendron*, owing to the large clusters of long-branched chains of predominantly continuous conidia borne on tall, erect, conidiophores. However, the presence of occasional bicellular spores (Fig. 2, H) justifies its classification as *Cladosporium*.

The only description of a *Cladosporium* parasitic on *Pisum sativum* that has been found in that of Cugini and Macchiati (1) given for the binomial *Cladosporium pisi*. These workers report no stem and foliage blight, an important phase of the disease under discussion, and the pod disease attributed to *C. pisi* coincides neither in description nor by illustration with the pod scab concerned here. The smaller spores, moreover, are described as mostly one-septate, with a constriction at the septum, and measure smaller than the species under consideration. Furthermore, the recent repetition by Curzi and Barbaini (2) of this Italian work leaves little doubt but that the condition dealt with in Italy is of nonparasitic origin and that *C. pisi* is a synonym for *C. herbarum* with no parasitism for *Pisum sativum*.

*Cladosporium album*, described by Dowson (3) on sweet peas, differs in that it is white and does not infect *Pisum*. Freedom of the conidia from echinulations distinguishes the present *Cladosporium* from *C. myriosporum*, reported on peas in Canada (4). Gardner (5) was unable to infect *Pisum* with *C. vignae*, and inoculations of cowpeas with the *Cladosporium* from peas have been unsuccessful. In addition, the sporophore and conidial measurements differ, being smaller in the case of *C. vignae*, and the color of colonies in artificial culture does not become so dark with age as does that of the pea *Cladosporium*. Apparently the *Cladosporium* concerned here is an undescribed species, for which the binomial, *Cladosporium pisicolum*, n. sp., is proposed.

***Cladosporium pisicolum*, n. sp.**

Colonies on potato-dextrose agar, at first velvety green, rapidly darkening to deep olive, and finally olivaceous black. Submerged mycelium a dull greenish black. Sporophores numerous, one- to several-septate, unbranched, often slightly bent at the septa, brown, measuring 75 to 225  $\mu$ . Conidia borne acrogenously in terminal clusters of branched chains, the spore size diminishing apically. Conidia light brown, the smaller ones ovoid to ellipsoidal, somewhat pointed at the ends, continuous, infrequently one-septate, averaging  $4.4 \times 8.7 \mu$ ; larger basal ones elongate, almost cylindrical, slightly pedicellate, continuous to one-septate, rarely two-septate, measuring 2 to 3 times longer. Parasitic upon *Pisum sativum* L., producing seedling blight, leaf and stipule spot, slightly sunken lesions on stem, petiole, peduncle, and pedicel, and pod scabs. Leaf spots 1 to 8 mm., tan with a narrow brownish border, roughly circular, interior or marginal. Pod scabs dark brown or black, irregular in shape, sunken or raised, sometimes penetrating the pod wall and invading the seed. The fungus is seed-borne and soil-borne, either source of inoculum infecting seedlings. Found in Monterey and other coastal counties in California.

**FUNGUS GROWTH IN RELATION TO TEMPERATURE**

The influence of temperature on the linear growth of the fungus was observed by growing the organism on potato-dextrose agar in duplicate at a series of temperatures. Average colony diameters in millimeters after 8 days were as follows for the given Centigrade temperatures: 0 at 3°, 10 at 8.5°, 17 at 11.5°, 29 at 18°, 41 at 22°, 27 at 25°, 19 at 27.5°, 3 at 31°, 0 at 34°. Repetition of the experiment gave no important differences from these results, which establish the minimum for growth between 3 and 8°, the maximum between 31 and 34°, and an optimum near 20 to 22° C.

**HOST RANGE**

A collection of pea varieties representative of market-pea culture in California was tested with certain other pea varieties and legumes for susceptibility to *Cladosporium pisicolum*. The results are shown in table 1. All plants were grown out-of-doors in glass chambers, which were open during the day but closed at night. Each variety was represented by 6 to 8 plants, and all were inoculated simultaneously with an atomized spore suspension. Repetition of varieties in table 1 indicates seed of different origin. An experiment performed at two different times, gave results of the same order both times.

Owing to the close similarity between the *Cladosporium* diseases of the pea and the cowpea, a number of varieties of cowpea, found susceptible to

*Cladosporium vignae* by Gardner (5), were tested<sup>5</sup> in duplicate trials for susceptibility to the pea *Cladosporium*, in addition to those listed in table 1. This collection included Iron, Large Blackeye, Early Red, New Era, Brabham, Clay, Groit, and, in addition, *Vigna sesquipedalis* Wight, the asparagus bean. No infection was obtained in any instance, although inoculations were made on very young seedlings as well as on unfolding secondary leaves.

All varieties of peas tested showed some degree of susceptibility to the fungus. Most resistant were the Canada field and Alaska types. No variety of cowpea, however, nor other legume tested, was found susceptible to the fungus aside from the exception noted in the case of *Vicia faba* in table 1, although more extensive tests may later reveal other susceptibles. The fact that the pea *Cladosporium* was not found parasitic on cowpea and on sweet pea and that *C. vignae* and *C. album* have been shown not to infect pea indicates that these fungi are not identical with the pea fungus.

#### SOIL INFESTATION

In certain of the coastal areas of California peas may be found growing almost every month of the year. The fungus has, therefore, no severe climatic conditions to endure nor long periods to withstand in absence of the host. Saprophytic growth of the *Cladosporium* is abundant upon old pea and other organic refuse and, in this manner, may be carried over in the soil from one planting to another. That carry-over of the fungus in the soil may constitute a particularly potent source of primary infection is illustrated in the following experiences. A fall crop of peas in a field near Watsonville was observed, in October, 1932, to be abundantly infected with the foliage blight of the *Cladosporium* disease. Near the first of March, 1933, the field was again visited. It had been cultivated but not planted since the removal of the peas in December, and a number of volunteer pea plants 3 to 4 inches high had emerged. Examination revealed that nearly every one of these plants was badly infested with *Cladosporium* and an occasional plant was killed. The leaves bore large interior and marginal lesions, and stem cankers were extensive and severe. More conclusive evidence, however, was obtained in a greenhouse trial. Diseased pods were incorporated in several pots of soil in October, which were allowed to stand outdoors through the winter. In February they were planted, in the greenhouse, with clean seed and bell jars inverted over the pots upon seedling emergence, to maintain a high humidity. Of 101 resulting plants, 29, or about 29 per cent, showed *Cladosporium* infection of the primary stipules or leaflets, or both (Fig. 2, C). Where healthy pods had been incorporated in the soil for controls, no infection of seedlings was obtained.

<sup>5</sup> The writer is indebted to Dr. W. J. Morse, Bureau of Plant Industry, U. S. D. A., for the collection of cowpea varieties used in these trials.

TABLE 1.—*Susceptibility of young plants of pea varieties and other legumes to infection by Cladosporium pisicolum*

Species	Variety	Susceptibility <sup>a</sup>	Species	Variety	Susceptibility
<i>Pisum sativum</i>	Morse's 200	+++	<i>Pisum sativum</i>	Improved Stratagem	++
"	Laxton's Progress	++++	"	Laxtonian	++
"	Laxtonian	++++	"	Hundredfold	++
"	Morse's Market	++++	"	Hundredfold	++
"	Alderman	++++	"	Senator	++
"	Alderman	++++	"	Little Marvel	++
"	Melting Sugar	++++	"	Blue Bantam	++
"	Stratagem	++++	"	Bruce	++
"	Laxton's Progress	++++	"	Resistant Alaskab	++
"	Hundredfold	++++	"	Alaska	++
"	Thomas Laxton	++++	"	Canada Field	+
"	Perfection	++++			
"	Wisconsin Early Sweet	++++	<i>Vigna sinensis</i>	Early Buff	0
"	American Wonder	++++	"	Columbia	0
"	Telephone	++	"	Victor	0
"	Dwarf Telephone	++	"	Progressive White	0
"	Gradius	++	"	Whippoorwill	0
"	Giant Butter	++	<i>Lathyrus odoratus</i>	Spencer Mixed	0
			<i>Vicia faba</i>	Large seeded	c

<sup>a</sup> + + + + = very severe, + + + = severe, + + = moderate, + = slight, 0 = no infection.<sup>b</sup> Resistant to the pea-wilt fungus, *Fusarium orthoceras* Appel and Wz. var. *pisi* Linford.<sup>c</sup> Moderate infection was obtained in the first trial, conducted under high greenhouse temperatures, but subsequent trials, out-of-doors under cool conditions, yielded no infection.

## SEED TRANSMISSION

Brown to black blemishes upon seed from infected pods early suggested that the fungus might be seed-borne. To test this possibility scabby Hundredfold pods were gathered from the cull bin of a pea packer and cured, and the seed harvested by hand. Many of the seed showed discolored spots where contact occurred with the infected pod wall before maturation of the seed. At the end of a 5-month period, during which the seeds were stored at room temperatures, they were planted in steam-sterilized sand in a clean humidity chamber. Of nearly 100 seed so planted, 5.5 per cent of the resulting seedlings developed *Cladosporium* spots on the first stipules or leaflets. No other lesions developed and the control plants remained free from disease. In another trial a selected collection of badly spotted seed from infected Alderman and Stratagem pods was planted in the greenhouse near a planting of healthy seed of the same varieties. No attempt was made to provide a humidity other than that prevailing in the greenhouse. Of 13 plants from the infected seed, 9 developed primary lesions on emergence, while no infection appeared on the 15 plants from healthy seed. These and other data demonstrated a high incidence of seedling infection from diseased seed.

That the fungus may be carried within the seed coats as well as on the surface was shown in a trial where spotted seeds from artificially infected pods were surface-sterilized and then allowed to germinate on agar in Petri dishes. Sterilization consisted in dipping the seeds in 85 per cent alcohol and then allowing them to stand in 1-1000 HgCl<sub>2</sub> for 5 minutes. After 2 washings in sterile water they were planted in potato-dextrose agar. An occasional seed, on germination in the agar, gave rise to a *Cladosporium* colony.

In about a week following the appearance of primary infections on seedlings grown from infected seed, secondary infections usually appear in connection with the continued unfolding of new foliage. This is true of plants outdoors or in the greenhouse where watering of the plants, especially when done in the evening, wets the foliage. It is evident that wind and rain are to be considered important agencies in secondary spread of the disease.

## ENVIRONMENTAL RELATIONSHIPS

As has been true with most other *Cladosporium* diseases, a high humidity is favorable, if not essential, to infection. This is particularly true of secondary spread of the pea *Cladosporium*. Primary infection from either infected seed or soil contamination is apparently initiated prior to emergence in the presence of the soil moisture affecting seed germination. Field infection, occurring late in the development of the plant

and resulting in pod scab, has generally been associated with a period of ocean fogs that for several days may bathe the plants continuously. The ocean breeze bearing the fog also provides for spore dissemination.

Although high humidity seems to be the most important of the environal factors for infection, a moderately cool temperature also is most favorable to the disease and is obtained hand in hand with coastal fogs. In fact, some growers have termed the disease "cold weather blight." The range of temperature most favorable to the disease appears to conform in general with that favorable for the pea plant, roughly, 60 to 70° F., although no controlled experiments on either temperature or humidity have been made. Attempts to inoculate plants held at temperatures much above or below this range have met with only partial success.

Other environal factors are of importance to the extent that they provide for the development of susceptible plant tissue. The young tender tissues are most susceptible, older tissues soon reaching a point where they become resistant to infection, a situation also reported for the cowpea disease. With all organs of the plant it is the appearance of new growth and succulent tissues with which *Cladosporium* infection is associated, thus confining new infection to the region of the growing points where new leaves are unfolding, to the blossom tissues, and to immature stems and pods.

#### HYPODERMIC POD INOCULATIONS

In order to observe the effect of the fungus when introduced into the interior of a growing pod, a suspension of *Cladosporium* spores in sterile distilled water was forced into pea pods of different varieties and ages upon growing greenhouse plants by means of a small hypodermic needle. At the same time a number of pods were treated with the hypodermic similarly, except that in some cases only air, no liquid, was forced into the pod, and in other cases sterile distilled water was injected. A small wound was, therefore, produced in each case but the material discharged was different. The results, observed 12 days later, are as follows:

Of 16 pods injected with air, 14 showed a proliferation of cells of the inner pod membrane for a space about  $\frac{1}{4}$  inch in diameter about the wound (Fig. 3, C). No other effects were observed, the remainder of the pod developing normally. Of 40 pods treated with water, 18 showed some proliferation of the pod wall of a mild type spreading some distance from the wound (Fig. 3, E). However, in the cases where the *Cladosporium* suspension had been injected, 42 out of 47 pods showed pronounced proliferation of the cells of the epithelial lining (Fig. 3, A, D) and, in addition, severe seed infection (Fig. 3, F); the remaining 5 showed seed infection, although the pods appeared to have been too mature at the time of inoculation to react with appreciable proliferation. The fungus could be found

fruiting not only in the patches of proliferations but also abundantly upon the seed (Fig. 3, A). A quantity of these seeds were dried and planted with a resultant very high percentage of seedling infection. The fungus suspension had also, in a few cases, been injected into the upper stem of growing pea plants, resulting in a cracking of the stem and a marked enlargement.

These tests in which the hypodermic needle was used illustrate two points. First, they demonstrate the active parasitism of the fungus, especially upon the green seed; and, second, they indicate that proliferation of the inner pod membrane may be incited by any one of several agencies, namely, a mechanical wound, free moisture, and presence of the fungus *Cladosporium*. Such proliferation, early described by Sorauer (7, p. 477), has frequently been noted in California coastal areas by the writer to occur in patches upon the interior of pods directly opposite pod infections of, not only *Cladosporium*, but, also, those of *Ascochyta pisi* and *Peronospora viciae*, and in instances where a developing pod has been punctured or injured while still on the vine and subject to a cool, humid environment.

The proliferation discussed here apparently consists of a hyperplasia of the epithelial lining of the pod cavity, resulting in a mat of hair-like growths (Fig. 3, B), and is not to be confused with the intumescences appearing upon the epicarp as described by Curzi and Barbaini (2). Following stimulation, cells of the inner membrane grow into the pod cavity, divide and redivide, and develop into long, usually curled hairs consisting of several cells, end to end, and containing chloroplasts. It is the production of these hair-like growths of pod tissue that results in the white matted patches, giving a felty appearance to the inside of the pod.

#### SUMMARY

A leaf, stem, and pod disease of the pea, *Pisum sativum*, is reported as caused by a hitherto undescribed species of *Cladosporium*, for which the binomial, *Cladosporium pisicolum*, n. sp., is proposed.

The disease has been found most abundant in peas grown for market trade in certain coastal areas of California.

Infection on the foliage appears as necrotic tan spots, roughly circular to irregular, delimited by a narrow line of dark brown. Stem lesions are dark brown to black and of varying shapes. Pod infection results in the formation of dark brown to black scabs of irregular shape with a sharply defined, black line of demarcation outlining them.

Under favorable conditions of high humidity and moderate temperature foliage infections occur within 3 to 7 days following inoculation. Only the young growing tissues are susceptible.



All varieties of *Pisum sativum* tested proved susceptible to infection, although in varying degrees. Cowpea, sweet pea, and the asparagus bean were not found susceptible.

Primary inoculum may be provided by soil infested with vine refuse from a previous crop harboring the fungus or through seed-borne infections of the fungus. Either may operate to cause the blighting of seedlings.

Hair-like proliferations of the inner pod membrane, resulting in white felty patches that extend into the pod cavity, may be induced by presence of the *Cladosporium* fungus in the pod. Other fungi, free moisture, and even mechanical agencies also appear capable of inciting internal pod proliferation, exclusive of *Cladosporium*, under the proper conditions.

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# CONTROL OF THE VERTICILLIUM WILT OF EGGPLANT<sup>1</sup>

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## INTRODUCTION

Eggplant wilt is one of the most serious vegetable diseases in Massachusetts. The losses in yield resulting from it have discouraged many market gardeners from continuing the culture of eggplant (Fig. 1). In view of the importance of the disease in this State, certain fundamental studies of the fungus and related field experiments were undertaken in the hope that some information of practical application might be obtained. The efforts to find feasible control measures have not been entirely successful, but certain information has been obtained that should be of interest.

## THE DISEASE

Wilt is prevalent annually in every eggplant field in Massachusetts; frequently the entire planting is a total failure. The disease usually appears in the field after the blossoms have set fruit. It rarely appears among the young plants in the hotbed or plant house. Either individual shoots or the entire plant may be killed, but the infected plants usually grow slowly during the early part of the summer and then revive in August and September. The growth of the fruit stops or is much retarded and the fruit may shrivel. Extensive field observations indicate that the vigor of the plant contributes nothing to its resistance to infection. The darkened color of the wood or vascular tissue, which is the most positive index of diagnosis, extends through the entire system of the plant, including the distal end of the fruit; but efforts of the writer and others (Carpenter (2), Haenseler (3)) to culture the organism from the seeds have been unsuccessful. Frequently, after wilt occurs, other organisms, notably *Sclerotinia sclerotiorum* (Lib.) Mass., aid in the destruction of the plants.

## CAUSAL ORGANISM

The causal organism has been designated *Verticillium ovatum* Berk. & Jack. (1), *V. albo-atrum* R. & B., and *V. dahliae* Kleb., without any agreement in the literature, and Rudolph (9) has suggested the name *V. albo-atrum* R. & B. var. *ovatum* B. & J.

The fungus is able to survive indefinitely in cultivated land, due, no doubt, to its ability to live as a saprophyte. At least, no instances of the control of eggplant wilt by long rotations with nonsusceptible crops have

<sup>1</sup> Contribution No. 176 of the Massachusetts Agricultural Experiment Station.



FIG. 1. Verticillium wilt of eggplant.

been observed in Massachusetts or reported elsewhere in the literature. In Massachusetts, the fungus is usually troublesome on okra, tomato, eggplant, potato, chrysanthemum, snapdragon, raspberry, blackberry, and maple. It may attack other crop plants and weeds, without, however, always producing the usual wilt symptoms. Aside from those hosts on which the disease is here observed every year, it is occasionally noted on peach, plum, rose, peony, and Japanese barberry. In view of the wide host range and the fact that affected plants wilt with the resulting loss of some or all of their yield, the fungus ranks as one of our most important plant pathogens.

TABLE 1.—*Relation of soil temperature to infection of eggplant with Verticillium*

Soil temperature		Plants infected	
°F.	°F.	per cent	per cent
55.4	77.	0.0	100.
59.	80.6	25.	50.
62.6	84.2	54.5	50.
66.2	87.8	66.	40.
69.8	91.4	72.7	18.1
73.4	95.	91.6	0.0

The persistence of the fungus in the soil, even in the absence of susceptible plants, and the fact that it is capable of existing as a saprophyte render its control exceedingly difficult. A second planting of eggplant on the same land, following a crop relatively wilt-free, always greatly increases the population of wilted plants and usually results in an eggplant failure. Even in rotations without susceptible crops for many years previous to eggplant, failures from wilt occur.

Observations strongly indicate that cultivated fields everywhere are infested with the fungus and that the soil in the field is the chief source of infection. This conclusion is entertained because the sterilization of the soil in plant beds has not reduced the prevalence of the disease in the field; also, plantings relatively wilt-free always occur even where the same beds are used year after year. According to Haenseler,<sup>2</sup> nearly all of the infection in New Jersey originates in the field, and sterilization of the soil in the plant bed is not considered worth while as a control practice. This has seemed to be the case in Massachusetts.

The fact that the causal organism inhabits the soil and invades the eggplant through its root system precludes any control by spray or dust treatments. For other diseases of a similar nature, control methods have proceeded essentially along the following lines; viz. (a) the development of resistant varieties, (b) soil disinfection or the modification of soil reaction with chemicals, and (c) soil fertilization. These methods were considered in the writer's efforts to find a control for the disease.

#### VARIETAL REACTION

The problem of developing resistance to eggplant wilt appeared of most interest and of greatest practical value but involved finding resistant individuals or varieties of the host for hybridization purposes. Some unsuccessful efforts in this direction have already been reported by Jagger and Stewart (7) who failed to find any resistance to the *Verticillium* fungus among a wide collection of strains and varieties of eggplant assembled from different parts of the world. Haenseler (4) reported that the Italian varieties, represented by the long purple types, are slightly resistant and that, in New Jersey, Florida High Bush and New York Improved endure the disease more satisfactorily than does Black Beauty, but none was sufficiently resistant to yield a paying crop on badly infested soils. Chupp<sup>3</sup> from field observations in New York reported Black Beauty as the most susceptible and Florida High Bush apparently resistant. In a more recent report of the study of the reaction of many species, varieties, and strains of

<sup>2</sup> Letter from Dr. C. M. Haenseler, New Jersey Agricultural Experiment Station, New Brunswick, N. J., October 11, 1932.

<sup>3</sup> Chupp, G. U. S. D. A. Plant Disease Rept. Suppl. 45: 144. 1925. May 1, 1926.

eggplant to the wilt fungus, Haenseler (6) noted that none showed special promise for use in developing resistant strains.

In view of the fact that this *Verticillium* fungus lacks specialization for any particular host or group of related hosts, any possible resistance among varieties of eggplant to the fungus would seemingly be exceedingly limited or entirely lacking. Past accomplishments in the development of crop plants resistant to fungus pathogens appear to have required some degree of host specialization as a prerequisite to success. Vavilov (10), as the result of his extensive studies of the distribution of immunity in connection with the specialization of parasitic fungi, concluded that the more general the field of receptivity to a given fungus, the less likely are there to be found immune or highly resistant varieties in the same species. This fact was clearly demonstrated in the writer's study of the reaction of many types of eggplant and related species assembled from many parts of the world. No evidence of significant differences in their reaction to the pathogen was noted. Since the fungus can infect a wide variety of plants regardless of those characters that define genera, families, and orders of plants, its specialization for certain varieties of a botanical species that differ only in minor and smaller characters is hardly conceivable. The study of the reaction of many varieties of eggplant demonstrates this fact and emphasizes the total lack of any material for selection or for hybridization purposes.

It was observed that some individuals each year failed to show wilt symptoms, but sections of the stems in the basal region of the plants frequently revealed the brown wood characteristic of infection. Some plants were late in their expression of the disease and others even escaped infection. Selection of such stock for resistance extending over a period of several years was, however, totally ineffective.

The early or heavy-fruited varieties, *e.g.*, New York Purple, showed the first signs of wilt, and late-cropping varieties, *e.g.*, Florida High Bush, always exhibited the disease later in the season. Light-cropping varieties and sterile plants either failed to show wilt or did not exhibit the characteristic symptoms until late in the season. Since the causal fungus is a vascular parasite, it interferes mechanically with the water stream of the plant and consumes water for its own growth. Its presence in the water vessels would obviously induce wilt during periods of maximum transpiration and in times of drought. This would explain the epidemic appearance of the disease when the plants are setting fruit and making their greatest amount of growth and would account for its belated appearance or absence among sterile or light-cropping individuals or varieties. This correlation in the manifestation of the disease with fruit setting and fruiting habits has not been considered by those who have reported degrees of resistance among varieties of eggplant, but it cannot be disregarded in efforts to breed or select for resistance to the disease.

## TEMPERATURE RELATIONS

Mycelium was planted on potato-dextrose agar in Erlenmeyer flasks. It was found that growth of *Verticillium* from eggplant grew at a temperature range of about 50°–95° F. and that the greatest amount of growth occurred at about 78° F. at the end of 72 hours. These results agree fairly well with earlier reports in the literature.

It was found that good growth of eggplant occurred at a soil temperature range of 77°–95° F., with optimum growth at 87°–92° F. The experimental plants were started in sterilized soil and transferred to inoculated soil in soil-temperature tanks in a greenhouse. Twelve plants divided among 4 containers were grown in each soil-temperature compartment. Infection was determined by the characteristic brown vascular ring as revealed in stem

TABLE 2.—Percentage of wilted eggplants as affected by chemical field treatments in 1930

Treatment	Rate per acre	Plants wilted		
		July 22	August 10	August 25
	<i>Tons</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Copper-Lime dust (20–80) . . . . .	4	73.6	91.8	97.9
Superphosphate . . . . .	3½	74.3	84.5	99.3
Aluminum sulphate . . . . .	4½ <sup>a</sup>	82.7	82.3	100.0
Winter rye, Fall 1929 seeding . . . . .		76.6	83.	99.3
No treatment . . . . .		90.9	90.9	97.2
Inoculated sulphur . . . . .	6 <sup>b</sup>	20.	60.	83.7
Sod 1925–1930. Corn prior to 1925 .		23.3	65.7	87.6

<sup>a</sup> 1½ tons applied in the fall of 1929; balance in April, 1930.

<sup>b</sup> 4 tons applied in the spring and fall of 1929; balance in April, 1930.

sections rather than by external symptoms alone. The highest percentage of infected plants was noted at 77° F. At the extreme temperatures, *i.e.*, 59° and 91.4° F., no external symptoms of disease were apparent, although cultures and sections of the main stem revealed the presence of infection. No infection occurred at 55.4° F. and 95° F. According to Ludbrook (8), *Verticillium dahliae* produced marked external disease symptoms at 86° F., but not at 89.6° F. Judging from the number of infected plants at each temperature, the optimum soil temperature for infection appeared to be within the range 73°–80° F. (Table 1).

The *Verticillium* wilt of eggplant is very prevalent and destructive in northern United States, but, judging from reports, occurrences of this disease on eggplant in the Southern States are rare or lacking. While the disease is definitely epidemic on the eggplant in the cooler regions of this coun-

try, there is nothing about the cardinal-temperature relations of the fungus that might suggest a satisfactory explanation of its restricted geographical distribution on this host.

In a field experiment, in 1929, with paper mulch, it was shown that the higher soil temperatures prevalent under paper caused an earlier appearance and a greater severity of wilt. Soil temperatures under paper at a depth of 2 and 6 inches, respectively, and at similar depths without paper, were recorded daily during June and July. The disease appeared 7 to 10 days earlier and was much more prevalent in the plot grown with paper than in the one without paper. Black mulch paper caused a greater and quicker growth of eggplant during June and early July, and the temperature records indicate that this accelerated growth was correlated with higher soil temperatures. Temperatures at 2 inches under paper were higher and nearer the optimum for infection than at 6 inches.

#### CHEMICAL APPLICATIONS

The spores of *Verticillium* are killed with sulphur, copper-lime (20–80), and naphthalene dusts. Aluminum sulphate, in concentrations ranging from 1–100 to 1–400, is lethal. These fungicides were applied to glass slides, and water-drop suspensions of spores were placed on the dry residue. The slides were incubated at 70° F. The lethal action of these chemicals on the spores suggested that some control might be expected from field treatments, although the practical limitations involved were fully realized.

In the season of 1929, naphthalene and copper-lime dusts were added to the hills in the field at planting time. Naphthalene was applied to each hill in quantities of 1½ oz. and 1 oz., and copper-lime dust in quantities of 2 oz. and 1½ oz. It was expected that the treated soil would be disinfected sufficiently to offer some control of wilt, but the results were disappointing. No control of the disease resulted.

Haenseler (5) found that soil pH of 6.0–7.0 was associated with badly wilted fields of eggplant and 4.0–5.0 with relatively small amounts of wilt. According to his studies, the growth of the organism in culture was greatly retarded at pH 4.2–4.5 and at pH 4.0 was extremely poor or lacking. Slight retardation in growth was noted in a pH range of 5.0–5.5, while optimum growth ranged from pH 6.0–8.0. On the other hand, the eggplant was found to tolerate pH 4.2–4.4 without injury. Field tests added support to his findings.

In the summer of 1929 the pH of the soil and the percentage of wilted plants in many fields of eggplant were determined by the writer. In general, a correlation existed between low pH (4.4–5.0) and good control of wilt. Fields with pH 5.0 or above did not show any consistent absence or presence of wilt, and, in view of the fact that high pH was associated with

high percentages of wilted plants, it was inferred that the prevalence of the disease in such fields was due to the lack of an inhibiting soil reaction. The determinations appeared to show that fields naturally acid, as the result of sod culture for several years, provided the most successful control of the disease.

In pure culture with potato-dextrose agar as a medium, growth of *Verticillium* showed marked retardation at approximately pH 5.0–5.2 when aluminum sulphate was used, and at pH 4.4–4.6 when inoculated sulphur



FIG. 2. Eggplant growing in soil at different pH values obtained with inoculated sulphur and showing the extreme low limit of tolerance of eggplant at pH 3.8–4.0 and the control of wilt below pH 4.6–4.8.

was used as the acidifying agent. A pH of 3.6 with inoculated sulphur and 4.0–4.1 with aluminum sulphate completely inhibited growth in culture.

Aluminum sulphate and inoculated sulphur were employed as soil-acidifying agents in a series of pot experiments to determine the lower limits of tolerance of the fungus and the eggplant. The plants were grown in containers holding 1 cubic foot of soil obtained from a naturally contaminated field. Increasing amounts of chemical were added and thoroughly mixed with the soil. The eggplant seedlings used in the tests were grown in



sterilized soil. In the aluminum sulphate series of pots, pH of about 4.7–4.8 impaired the growth of eggplant. No infection occurred below pH 5.0 and no control was obtained above pH 5.5. According to the results a pH range of 4.8–5.5 obtained with aluminum sulphate in the field would be expected to offer some control of the disease without injuring the eggplant. When inoculated sulphur was employed, a pH of 3.7–4.0 and less impaired growth (Fig. 2); excellent control of infection was obtained at 4.0–4.2, and no control at 4.8. Thus, a range of pH 4.0–4.8 in the field with sulphur would be expected to offer control without impairing the growth of the host. Aluminum sulphate gave more consistent control of wilt at the low values than sulphur and, therefore, appeared to be more promising. Undoubtedly, the toxicity of the aluminum, aside from the pH, was an important contributing factor. The result with sulphur confirms the report of Haenseler (5), and better agreement could not be expected. Such narrow ranges in pH would be exceedingly difficult to obtain in practice, however, and likely to be attended either with no control of the disease or with injury to the plants sufficient to render them unproductive.

In April, 1929, a plot of land on which eggplant had been grown for 3 years previously was treated with sulphur at the rate of a ton per acre. The soil of the nontreated plot showed a pH of 6.2. In July the treated plot had a pH of 5.5 and in September of 4.8. No control of wilt was obtained during the crop season of 1929, due apparently to the fact that at planting time the soil was insufficiently acid to exert any appreciable inhibiting effect upon the causal organism.

Eggplant was again grown on these plots in 1930. Comparisons were also made of the effect of different chemicals (Table 2). Copper-lime dust (20–80) was applied at the rate of 4 tons per acre, and superphosphate at the rate of 3½ tons per acre in April, 1930. A heavy crop of winter rye was plowed under in early May and dressings of aluminum sulphate and inoculated sulphur supplemented applications of the previous fall and the spring of 1929. Another plot in an adjacent field was in sod from 1925 to 1930

TABLE 3.—Percentage of wilted eggplants and corresponding field treatments and soil pH values, July 20, 1931

Material	pH value	Wilted plants	Plant growth
		<i>per cent</i>	
Aluminum sulphate .....	5.0–5.6	65	Good
Fall 1929, rye cover crop .....	5.8–6.2	73	“
No treatment .....	5.0–5.4	63	“
Inoculated sulphur plot A .....	3.8–4.3	22	Poor
Inoculated sulphur plot B .....	3.1–3.9	0	Very poor

and in corn for several years prior to this period. Poor growth and marked dwarfing, but a delay in the appearance of the disease, prevailed in the sulphur plot, and many of the plants died. No benefit was shown from turning under a thick stand of winter rye. Eggplant after sod showed the best growth. In this plot the symptoms of the disease were delayed and the yield was best in spite of the high percentage of infection. In the other plots wilt was general and no benefit resulted from the treatments.

These same plots were seeded to winter rye in the fall of 1930. Growth of the rye was completely inhibited in sulphur plot B and in sulphur plot A there was but a scant growth of it. In the other plots the rye made an even, strong growth. Wilt was well controlled in sulphur plot A, but the reaction of the soil seriously affected the growth of eggplant. In sulphur plot B no infected plants were detected, but growth was extremely poor (Table 3).

Another attempt to control wilt with sulphur and aluminum sulphate in the crop season of 1931 proved unsatisfactory. Applications of inoculated sulphur and aluminum sulphate in April, at the rate of a ton per acre, failed to control the wilt.

Haenseler (5) reported that after 4 years of sulphur treatments and after at least 2 years of extremely acid soil, where the pH was lowered to 4.2, 4.4, and 4.8, as great as 90 per cent, 90 per cent, and 100 per cent of the plants, respectively, showed wilt, although the advent of the disease was much delayed. It may be concluded that artificial soil acidification is not a practical means of control.

Our results, however, indicate the lack of need of lime for eggplant and the desirability of selecting fields with extremely low pH values. Observations and the experience of growers also indicate the undesirability of cropping twice in succession to eggplant, regardless of the soil reaction. Planting each year on new areas of old sod land that is naturally acid has offered the most economical and effective method of combating eggplant wilt.

#### SUMMARY

No varieties of eggplant showing any degree of resistance to the *Verticillium*-wilt pathogen have been found.

The fungus is not seed-borne.

The minimum prevalence of wilt was associated with culture on old sod land of pH values below 5.0. Planting each year on new areas of old sod land appears to be the only means of control.

In the greenhouse no infection occurred at soil pH values below 5.0 when aluminum sulphate was used as an acidifying agent, and only small amounts developed at a pH of 4.0-4.2 when inoculated sulphur was employed. Aluminum sulphate gave more consistent control of wilt at the low values than sulphur.

Field applications of aluminum sulphate and sulphur are of no practical value.

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# ROOT-KNOT AND OTHER NEMATODES ATTACKING RICE AND SOME ASSOCIATED WEEDS<sup>1</sup>

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## THE NEMIC PARASITES OF THE RICE PLANT PREVIOUSLY REPORTED

The rice plant is subject to a number of nemic diseases. As early as 1902 Breda de Haan (1) described a disease of the rice plant from Java called "omo mentèk" or "omo bambang," which he thought was caused by *Tylenchus oryzae* Breda de Haan 1902<sup>2</sup>, the rice nematode, a relationship later disclaimed by the same author (2). In 1913 and 1919 Butler reported another disease of the rice plant from India, with *T. angustus* Butler 1913 as its causative agent (3 and 4). The present paper adds to these two nemic enemies of rice two new ones, caused by widespread and very pathogenic parasites, *Heterodera marioni* (Cornu 1879) Goodey 1932, the root-knot nematode, and *T. pratensis* deMan 1880, the meadow nematode, together with a few other species whose pathological significance is not known or that must be considered parasites of secondary character.

Breda de Haan mentioned a species of *Dorylaimus* and two species of *Cephalobus* (one of about 0.9 mm. length and 0.017 mm. width and another 0.45 mm. by 0.021 mm.). The *Dorylaimus* he reported as rare and as occurring only in parts already beginning to decay. The two *Cephalobi* may be identical with the two species (*Cephalobus elongatus* deMan 1880 and *C. persegis* Bastian 1865), which were observed by the present writer in rice-plant roots from Arkansas associated with the forms listed on p. 917.

In 1931 Imamura (6) published an extensive study of the nemic fauna of the soil of a paddy field in Japan, without, however, considering the parasites of the rice plant itself or referring to any relationship between the nemic soil fauna and the plants. Of the numerous species recorded by this Japanese investigator, only two, *Cephalobus elongatus* and *C. persegis*, were found also by the present writer, as stated above, in the roots of the rice plant.

The parasitic nematode fauna of the rice plant as known at present, therefore, comprises the following forms:

<sup>1</sup> The material for the present study is that referred to by Dr. E. C. Tullis in a paper published in this issue of PHYTOPATHOLOGY. The illustrations were prepared by Mrs. Josephine F. Danforth; technical assistance was given by Miss Edna M. Buhner, Mrs. Civella A. Brooks, and Mrs. Florence M. Albin, all of this Division.

<sup>2</sup> The generic name *Anguillulina* has now replaced *Tylenchus* in general usage. See Baylis and Daubney: A Synopsis of the Families and Genera of Nematoda. London. 1926.

*Tylenchus oryzae* Breda de Haan 1902

*Tylenchus angustus* Butler 1913

*Tylenchus pratensis* deMan 1880

*Heterodera marioni* (Cornu 1879) Goodey 1932

*Aphelenchoides parietinus* (Bastian 1865) Steiner 1932

*Cephalobus elongatus* deMan 1880

*Cephalobus persegnis* Bastian 1865

*Acrobeloides bütschlii* (deMan 1884) Thorne 1925

*Acrobeloides cornis* Thorne 1925

*Tylenchus oryzae* is insufficiently described; no figures are available. Goodey (5) places it under the species *inquirendae*. A restudy of the species and also of its relationship to the rice plant is needed. In its mode of living in the roots of the host it most closely resembles *T. pratensis*, but the morphological characters, scantily given by its author, prove the form to be different. The spear is said to be knobbed, the median bulb well developed ( $16\ \mu$  wide), with the excretory pore opening ventrad and slightly back of it ( $75\ \mu$  behind anterior end). The tail is described as more or less pointed, ending sometimes with a spine-like, often eccentrically placed, terminus. The average length of the female is given as 1.5 mm. by a width of 0.043 mm., its maximum length as 1.82 mm.; position of vulva as at 63.5 per cent of the total length, that of the anus as at 93.7 per cent; body width at median bulb as  $42\ \mu$ ; spear  $19\ \mu$  and its knobs  $3.3\ \mu$ ; the oval-shape eggs  $102\ \mu \times 26\ \mu$ . The male is said to be slightly smaller than the female and the freshly hatched larva to measure 0.5 mm. by 0.01 mm. width.

*Tylenchus angustus*, in contrast to the aforementioned form, is a well-characterized species, and its parasitic relationship to the rice plant on which it produces a disease of stems and leaves called "ufra" is beyond doubt. For its description we refer to the two papers by Butler.

#### OBSERVATIONS ON THE DISTRIBUTION OF THE VARIOUS NEMATODE SPECIES IN AN INFESTED RICE ROOT

Only a few roots of plants from the infested rice field were at our disposal, but there was more material grown under greenhouse conditions in flats of root-knot-infested tobacco soil. In the original plants from the rice field only the root-knot nematode was found. The plants from the experimental flats, however, harbored a variety of nemie forms, of which, in addition to the root-knot species, *Tylenchus pratensis* must be considered as a very serious pest. Since in this latter instance the inoculum came from root-knot-infested tobacco roots and soil around them, it is assumed that all species found on the roots from the experimental flats had previously been

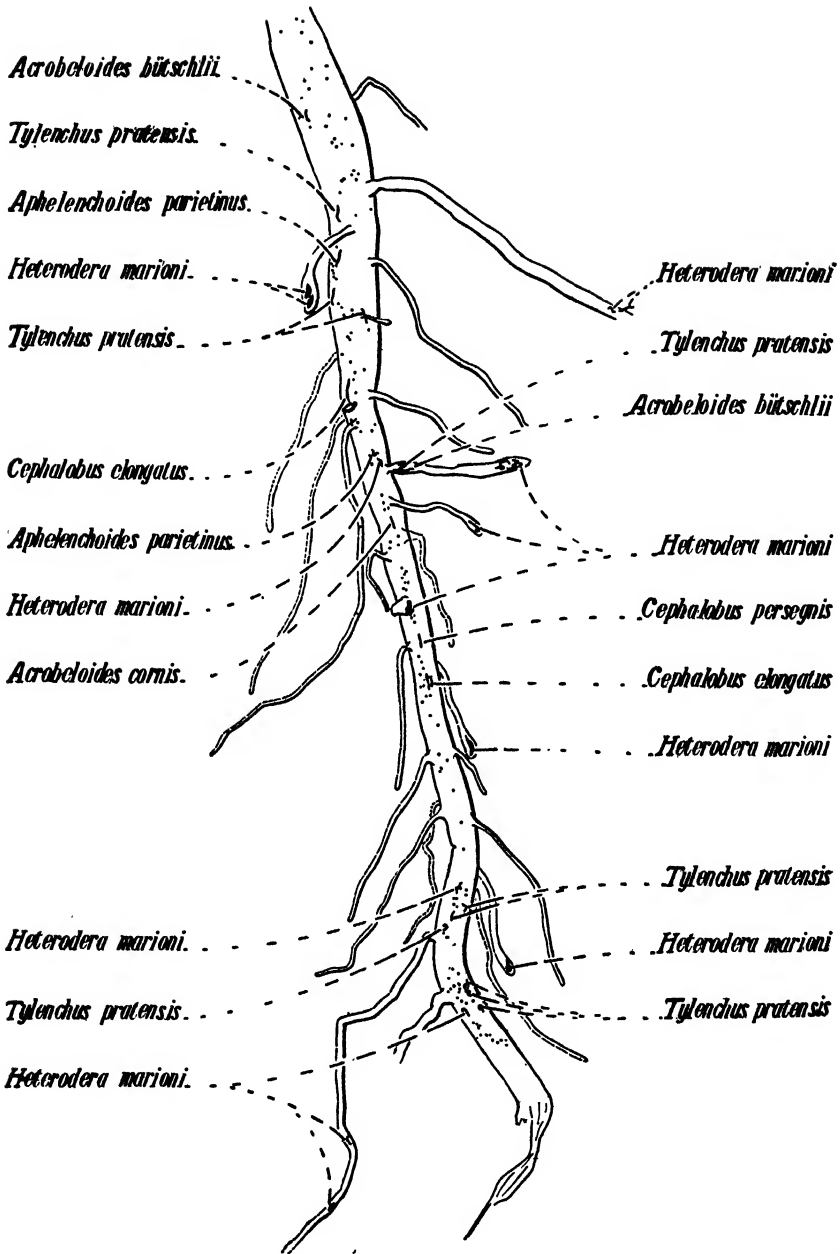


FIG. 1. Portion of root of a 9-day-old rice seedling showing distribution of the various nematode parasites through the roots. The top portion of the root is flattened. All nematodes infesting the roots are drawn in their natural location and position, and as nearly as possible to the same scale as the root itself.  $\times 8$ .

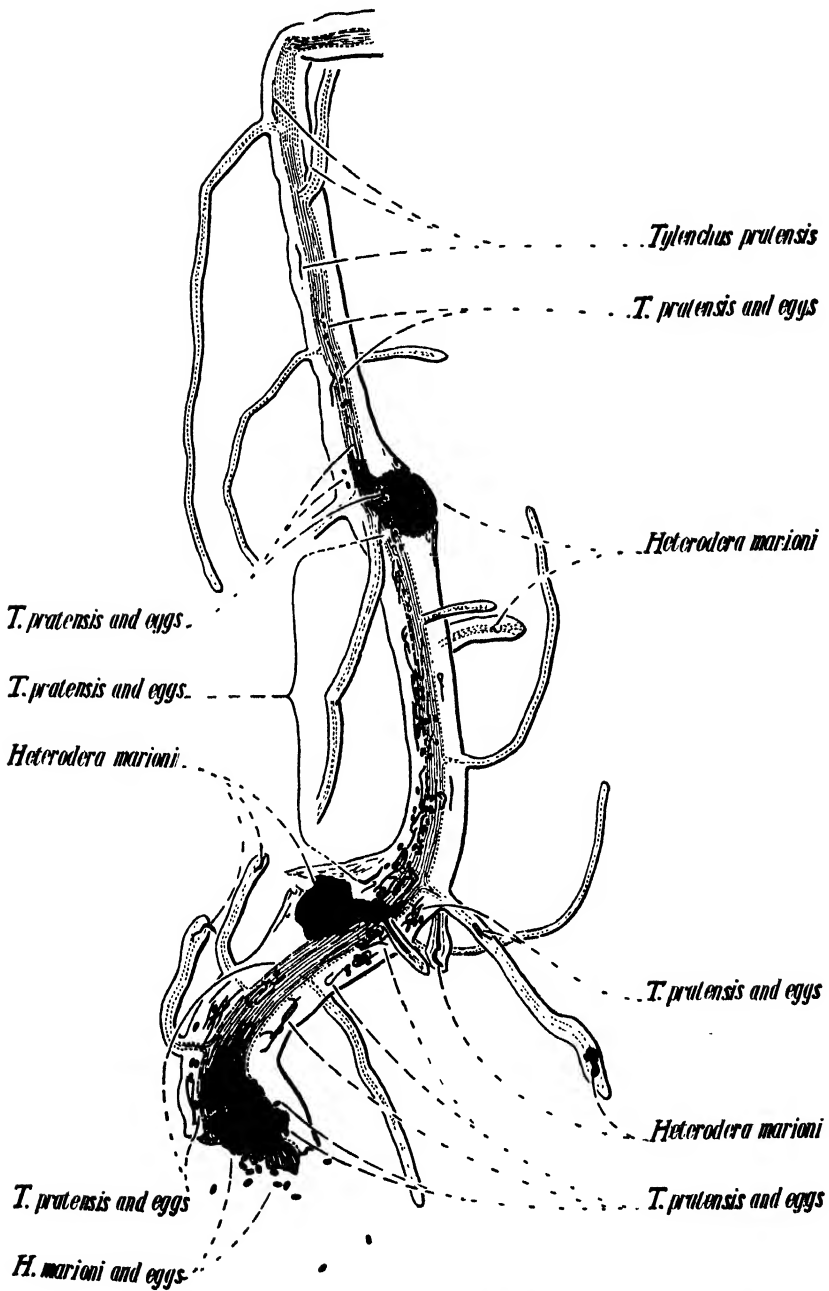


FIG. 2. Piece of a 5-week-old Blue Rose rice seedling root showing a heavy mixed infestation by the root-knot nematode and the meadow nematode (*Tylenchus pratensis*).  
 X 15.

feeding on tobacco plants. As to the significance of *Tylenchus pratensis* as a disease agent in tobacco roots, we refer to a note by the present author (8) and another by Lehman (7). Rice plant seedlings have very transparent roots. Nematodes parasitizing them can be seen clearly without special technique.

To show the location and distribution of these nemas through the roots, a piece of root, about 21 mm. long, is sketched in figure 1. Each nema found there is drawn at the location where observed, in about the same scale as the root itself. The black spots represent chytrids. Eggs were not present in this root portion. The total of nemic specimens observed was 37, belonging to the various species in the following quotas:

<i>Heterodera marioni</i> .....	18
<i>Tylenchus pratensis</i> .....	11
<i>Aphelenchoides parietinus</i> .....	2
<i>Cephalobus elongatus</i> .....	2
<i>Cephalobus persegnis</i> .....	1
<i>Acrobeloides bütschlii</i> .....	2
<i>Acrobeloides cornis</i> .....	1
	—
	37

The distribution of the various forms through the roots shows that *Heterodera marioni* has a preference for the root tips. This form also is the only one to enter the vascular tissues; all other species remain in the parenchyma of the cortex. *Tylenchus pratensis* has a tendency to form nests, that is, to heap together. This tendency is more plainly seen in figure 2.

#### THE ROOT-KNOT NEMATODE IN THE RICE ROOTS

It is evident that the rice plant suffers more than numerous other hosts from an attack by this nematode for the following reason: As may be seen from figures 2, C and 3, A in Tullis' paper, the parasitized root tips go blind, and, whereas other plants may counteract and repair the effects of the parasite through additional tissue growth, rice-plant roots exhibit only a reduced faculty for such repair. A comparison of figures 2 and 3, *a* illustrates best the two situations. Figure 2 plainly shows that the enlarging female body pushes the parenchyma of the root cortex aside until the nematode almost reaches the surface or actually breaks through the root epidermis. Thus, through lack of growth of reinforcing tissues, a weak spot develops at which, under tension, the root will break, as the figure so well demonstrates. On the other hand, it is plain that such a situation also is favorable to additional infection by fungi, bacteria, etc., and so, to regular decay, and in-



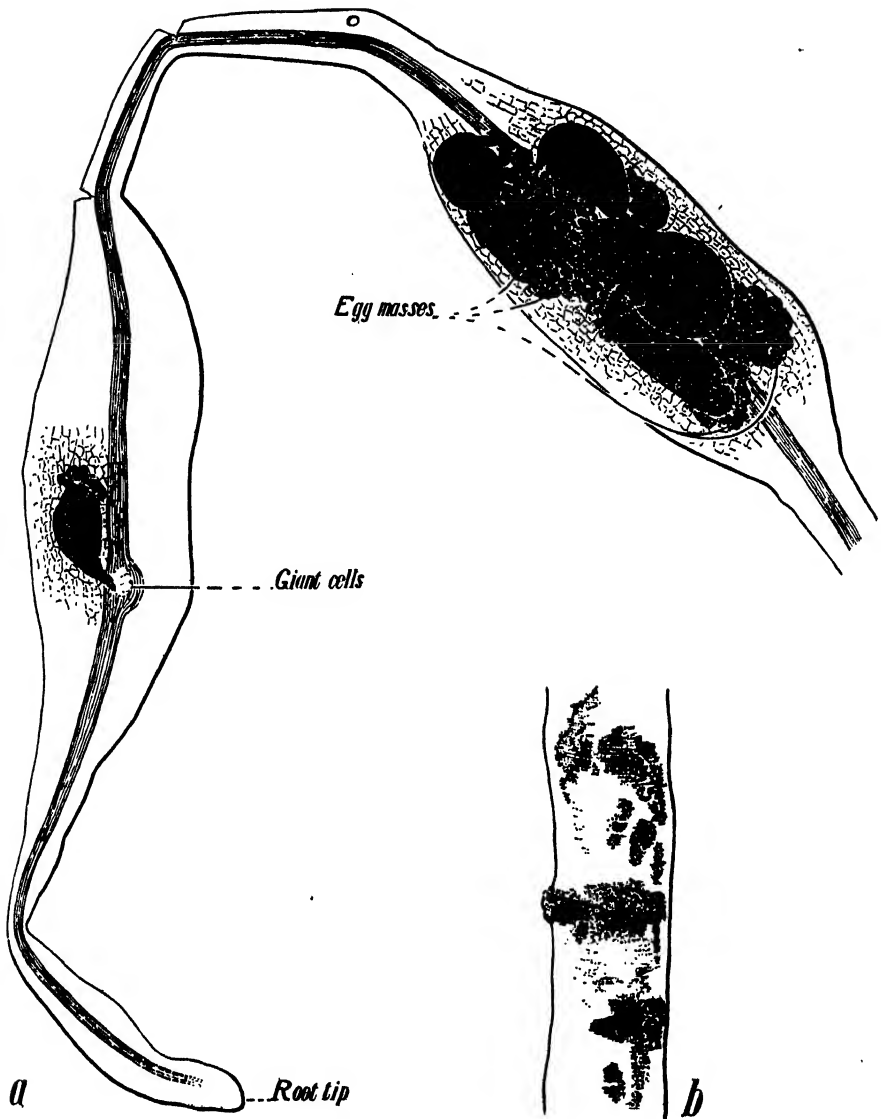


FIG. 3. a. Piece of *Echinochloa crusgalli* root infested with *Heterodera marioni*.  $\times 38$ . b. Lesions on a piece of root of *Amaranthus spinosus* harboring *Cephalobus longicaudatus*.  $\times 7$ .

creases the pathogenicity of the nema, especially as compared with the ease of figure 3, a. Occasionally specimens are seen that protrude from a root when still in a larval stage (Fig. 4, b). In this case the root apparently did not have strength enough or the necessary regenerative power even to grow

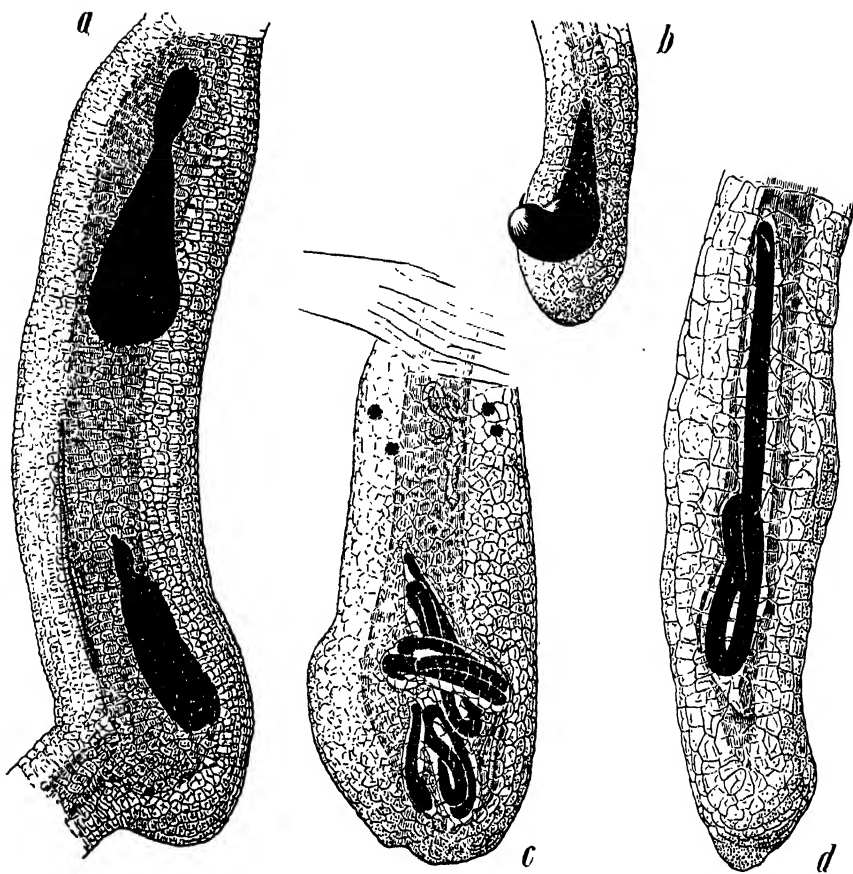


FIG. 4. Root tips of 9-day-old rice seedlings infested with *Heterodera marioni*. *a*. With side root on opposite side of original infestation, which apparently caused the primary root tip to go blind. *b*. Showing larva of the nema partly protruding from the root. *c*. Infestation with seven *H. marioni* specimens, four of which are larval males. *d*. With infestation by a single, but large, larval male. *a*, *b*, *c*  $\times 73$ ; *d*  $\times 94$ .

around this larva. In the rice plant the root-knot nematode takes the usual position within the root, penetrating the vascular tissue with its head and remaining with the posterior portion of the body in the root cortex. The number of the giant cells is 5 to 6, an unusually large number, but the individual cell is rather small. The giant cells, as far as seen, are always transformed plerome cells. If the number of the latter be increased at all, the increase is certainly not very great (Fig. 5).

As usual, the main point of entrance of the nematodes into the root system of the host is the root tip. It is perhaps because of this that almost all specimens have in the tissue a centripetal position, with the head end toward

the plant. Rarely are specimens seen in the centrifugal position, with the head toward the root tip. This is remarkable because, in *Echinochloa crus-galli* roots (Fig. 3, *a*) this latter inverse position of the parasite seems to be the rule.

The root-knot galls on the rice plant are, judging from the present material, mostly terminal galls, the root tips apparently being checked in further growth by the infestation and thus becoming blind. Of special interest in this connection is the case shown in figure 4, *a*. A comparatively strong

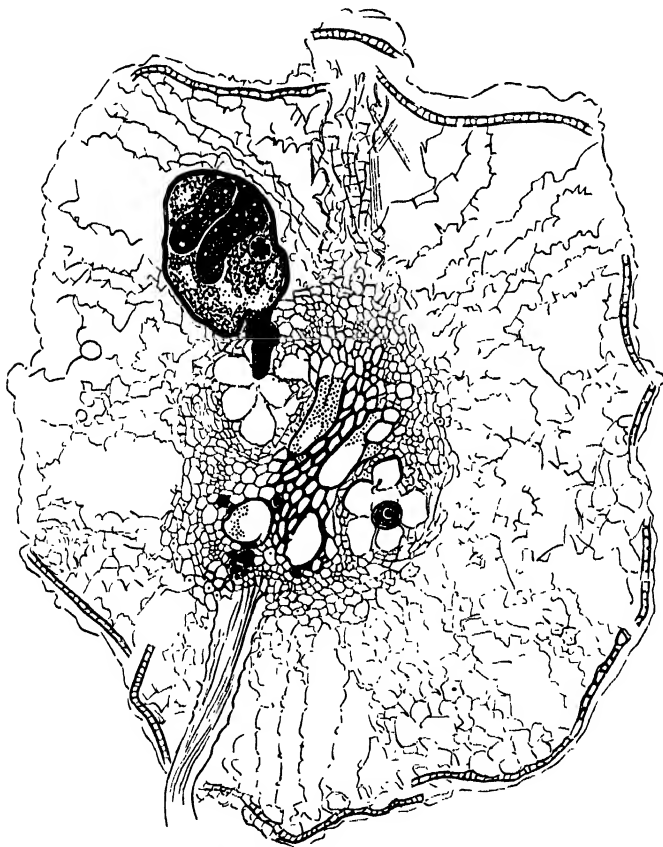


FIG. 5. Cross section through a partly macerated rice root showing the giant cells and the position of the root-knot nema in the root tissue.  $\times 108$ .

root was attacked by 2 nemas that fixed themselves behind each other on the same side of the vascular cylinder. The primary root tip was thus checked and went blind. However, since the vascular cylinder of the other side was not interfered with and remained strong enough, it grew on and developed a lateral root slightly subterminal to the primary root tip. The figure also shows that the infested root portion was entered again by young larval

specimens that moved to the unaffected side of the root but had not yet fixed themselves in the vascular tissue.

Figures 1 (of Tullis' paper) and 4, *c* (of this), raise the question as to what number of root-knot nematode larvae may enter a given root portion and settle in it. Is space the only deciding factor or are food and other physiological conditions also determining points? Figure 4, *c* shows a root tip with a total of 7 nemas, 4 of which are almost full-grown males. This root is from a 9-day-old seedling, taken from the soil, washed, and sent from Arkansas to Washington. While this handling in all instances killed the female larvae throughout the roots, male larvae apparently developed further, in spite of the complete suspension of growth of the host and the resultant check in the flow of the nutritive sap. This seems to indicate that the males, during their metamorphosis (if such a term may be applied to the last moult of the root-knot nematode male), do not take food and that food shortage may induce the larvae of a given age to develop into males (compare Jocelyn Tyler (10)). The case of figure 4, *d*, too, supports this latter view. It is of interest that all the male specimens within their moult took an inverse position, the tail end of the male being in the head portion of the larval moulting case. Is this accidental or the result of special conditions? Figure 4, *d*, as compared with figure 4, *c*, also shows that the size of the male varies, partly because of the amount of available food, which of course is less in highly infested root portions. From the present observation it is apparent that in rice roots the time needed for the development of the larva from the moment it fixes itself in the tissue to the egg-producing stage is about 4 weeks. The adult female measures about 0.560 mm. in length and 0.536 mm. in width. The size of the eggs was  $42\ \mu \times 96.6\ \mu$ —98.7  $\mu$ .

#### TYLENCHUS PRATENSIS AS A PARASITE IN RICE ROOTS

It is the first time that this nema is recorded as attacking rice roots. In this case the infestation apparently originated from tobacco. It is not known if this species would survive and persist in a rice field if under water. *Tylenchus pratensis* is found only in the parenchymal tissues of plant roots and the rice plant is no exception to this rule. In the present case only females and larvae were observed. Figure 6, *b* shows a group of embryonated eggs of apparently the same age deposited close together. Inasmuch as nemas were seen in this region of the root, it is thought that a single female deposited these eggs and afterwards left, according to the vagrant habit of this species. If these eggs had been left to develop, they would have given rise to a "nest" of *Tylenchus pratensis*. The seedling plant in whose roots these eggs were found was 9 days old when taken from the flat. It was cleaned of soil and sent from Arkansas to Washington, D. C., where the material was then fixed. Within this period of time (about 2 weeks) the eggs, which are deposited unsegmented, developed to the embryonate stage (Fig.

6, b). Figure 6, a shows a female that apparently had produced the 6 eggs. *Tylenchus pratensis* eggs, which measure  $20-21\mu$  by  $60-68\mu$ , are often slightly incurved, especially in certain cleavage stages. Because of their much greater length than width the 4-cell cleavage stage shows the cells still in a serial arrangement (Fig. 6, c). In the root these eggs have regularly a more or less axial position. In no instance was an egg seen in a

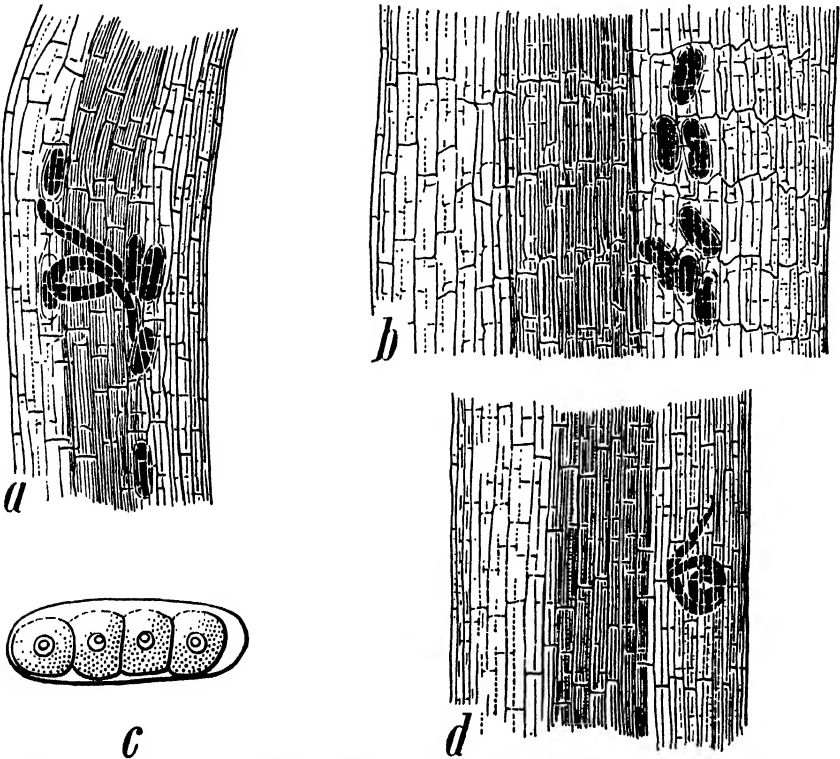


FIG. 6. a. Female *Tylenchus pratensis* and 6 deposited eggs in root of rice seedling. b. Embryonated eggs of *T. pratensis* in root of rice seedling. c. Egg of *T. pratensis* in 4-cell cleavage stage. d. *Aphelenchoides parietinus* specimen in healthy root parenchyma of rice seedling. a, b,  $d \times 110$ ; c  $\times 470$ .

strictly transverse position. Whether this is the result of the character of the root cells and the type of tissue or of the mode of deposition remains an open question.

There is a fundamental difference between a parasitism as effected by the root-knot nematode and that by *Tylenchus pratensis* or other forms here mentioned. The former is a sedentary, fixed parasite; the latter are vagrant forms. The root-knot nematode transforms the host tissues through injections. Giant cells—galls—are formed by the host as a reaction to these injections. The giant cells are nectarials—food cells. *Heterodera marioni*

has, therefore, extra oral, perioral digestion. *T. pratensis* is very different in this respect; producing no galls or giant cells, it in no way influences the host tissues to specific reactions. Its digestion is still decidedly intestinal. Apparently, the plasma of the parenchymal cells is ingested by this species, but a direct proof of this is not yet available.

Némec pointed out that the larval *Heterodera* uses the intercellular spaces to move through the tissues. Apparently, this habit has some relation to the fact that this nematode needs an otherwise healthy root that is able to react, so that the giant cells may be formed. Only a root that is undisturbed in other respects, the tissues of which are unharmed, may do so. It seems, therefore, to be in the interest of the parasite not to harm the tissues more than necessary; hence, apparently, the migration by means of the intercellular spaces. The other parasitic forms here mentioned are quite different in this regard. They force themselves through cell walls and damage or destroy cells and tissues in other ways. These latter nematodes are thus mechanically destructive and, in this respect, different from the root-knot form, which primarily only weakens a plant by extraction of food. Of course, secondarily, additional damage is done to certain hosts by interference with the flow of sap in the vascular system and by breaking up the tissues through the expansion of the parasite's body. (Fig. 2). A parasitism like that of *Heterodera marioni* is less likely to produce general decay than that of *Tylenchus pratensis* and other forms of the vagrant type.

#### APHELENCHOIDES PARIETINUS AND THE OTHER FORMS OF MINOR SIGNIFICANCE IN RICE SEEDLINGS

*Aphelenchoides parietinus*, *Cephalobus elongatus*, *C. persegnis*, and *Acrobeloides bütschlii* are forms of world-wide distribution. For none has the exact status as a plant pathogen been worked out. Although best regarded as secondary disease agents, it would be wrong to consider them only related to decay, for all these species also are found in healthy tissues, where decay has not yet set in—the situation in the roots of our rice seedlings. The *Aphelenchoides parietinus* sketched in figure 6, *d* is plainly shown within otherwise structurally healthy parenchymal tissue of the cortex. Of course, the root, part of which was sketched, already harbored numerous parasites that interfered with the normal root functions, creating physiologically unhealthy conditions and, subsequently perhaps, an incitement for these secondary parasites. Undoubtedly they had only begun their phase of destruction, the specimens observed being mainly in the larval stage.

Unlike the aforementioned species, *Acrobeloides cornis* probably is of restricted distribution, observed heretofore only in the Rocky Mountain region. The specimen seen agreed with those specimens described by Thorne (9), except that the tail end was more pointed and not so obtuse.

THE ROOT-KNOT GALLS OF *ECHINOCHLOA CRUSGALLI*

*Amaranthus spinosus* and *Echinochloa crusgalli* were associated in the field with the infested rice plants. Only the latter of the two weeds was attacked by the root-knot nema. The reaction of this host plant to the parasite differs somewhat from that of the rice plant. The galls are larger and neatly oval or spindle-shape (Fig. 3, *a*). In no instance did the tissue crack to give space to protruding female nematodes. A comparison of infested rice and *E. crusgalli* roots shows the latter better fitted for this parasitism. The roots form "healthier" galls, if this expression be permissible; their tissues are seemingly less interfered with. In the rice roots, as mentioned above, the parenchyma of the cortex appeared unable to make the necessary supplementary growth to form proper galls. In *E. crusgalli* the nemas have most often an inverse position in the root, indicating an entrance by the parasite in a nonterminal section of the root, whereas a "normal" position would result from an infestation by way of the root tip.

In regard to the formation of giant cells in *Echinochloa*, it may be pointed out that only 3 or 4, rarely 5, are formed. In total preparations of the roots they often show as a light, more transparent spot, immediately surrounding the head end of the nema (Fig. 3, *a*). As may be seen from the same figure, considerable masses of eggs are pressed into the parenchymal tissue of the cortex immediately surrounding the vulva. A gelatinous substance is found around the eggs and the whole mass of both is often larger than the female nema. But an egg sac, such as described by other authors, was not seen.

## AMARANTHUS SPINOSUS AND ITS NEMIC PARASITES

Although growing in close association with the root-knot-infested rice and *Echinochloa crusgalli* plants and known to be a host of *Heterodera marioni*, it was rather surprising to find *Amaranthus spinosus* not attacked by this nema. Probably this is a case of a root-knot population with a restricted host range that excludes the amaranth. However, the roots of the present plant harbored various other nemas, some of which are of special interest, as may be seen from the following list:

*Rhabditis* sp. 4 females; 4 larvae.

*Cephalobus striatus* Bastian 1865. 1 male; 3 larvae.

*Cephalobus elongatus* deMan 1880. 1 larva.

*Cephalobus longicaudatus* Bütschli 1873.

An apparently new genus of the Cephalobinae. 6 males; 3 larvae.

*Plectus rhizophilus* deMan 1880. 4 larvae.

*Tylenchus multicinctus* Cobb 1893. 5 larvae.

*Tylenchus dubius* Bütschli 1873. 1 male; 1 female; 3 larvae.

*Tylenchus leptosoma* deMan 1880. 1 female.

*Aphelenchoides fragariae* (Ritzema Bos 1891) Christie 1932. 1 female.

Three Dorylaims belonging to 2 different species.

*Aphelenchoides fragariae* was seen in a single specimen of undoubted character. Its appearance in these amaranth roots is rather surprising and might be wholly accidental.

*Tylenchus multicinctus* is a form of wide geographic distribution, especially in tropical and subtropical regions, but little is known as to its exact significance as a plant pathogen. *Tylenchus dubius* also belongs in this category. The Cephalobus were represented by 4 different species, *Cephalobus striatus* and *C. elongatus* being well-known decay associates with decided pathogenic characters of their own. *C. longicaudatus* is a rather rare form. It was found in reddish brown lesions (Fig. 3, b) on only one of the amaranth roots. However, its nature as a causative agent of these lesions was not checked by experiment and remains questionable. Another Cephalobus was found that apparently represents a new species and genus and will be described elsewhere.

*Plectus rhizophilus* and also the Dorylaims mentioned are obscure in their significance in the root tissues where found.

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# EXPERIMENTAL PRODUCTION OF CROWN GALL ON OPUNTIA<sup>1</sup>

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(Accepted for publication November 13, 1933)

In an earlier report the writer (3) called attention to the fact that young plants of the tree cactus, *Carnegiea gigantea*, respond to inoculations with *Bacterium tumefaciens* by producing typical crown galls comparable to those of the red beet and other garden plants generally used in experimental studies of this disease. It was pointed out that Smith (5) was able to produce a growth in one of four varieties of *Opuntia* that regressed, a phenomenon common in animal tumors but not reported for plants.

As far as could be determined by a survey of the literature, crown gall on other Cactaceae has been unknown. Brown and Evans (1) report that galls on the giant cactus, *Carnegiea gigantea*, have been known to the senior author for 20 years and have been regarded by him as crown galls. These structures occur on the stem and root of the plant and attain a diameter of 2 or more feet on the stem, and even larger overgrowths have been found on the root. Culture of the crown-gall tissue yielded, according to these authors, a uni-to-bi-flagellate bacterium, measurements of which correspond to those of the crown-gall organism. Unfortunately, the authors fail to give these measurements, for upon them apparently, rests their only evidence that they have isolated the tumor-producing bacterium. Their inoculations have not yet resulted in the development of a single crown gall.

There is evidently no reason to suspect the absence of crown gall in Arizona, as Lockwood's (4) interesting account of the early agricultural activities in this country relates that the Mission Gardens were stocked with fruit trees, common hosts to the crown-gall organism. Proof of the existence of this disease in nature on the Sahuaro has not yet been entirely established.

## METHODS AND MATERIALS

The apparent interest in these very unique plants, the Cactaceae, and the inability, up to the present, to produce tumors on the *Opuntia* species, have led me to repeat inoculations on freshly rooted joints of the same group of *Opuntias* reported in my previous communication. (*Opuntia Keyensis*, *O. Dillenii*, *O. stricta*, and *O. Linderheimeri* and a large number of plants produced from joints of a species of Floridian *Opuntia*.)

The methods of inoculation used in these tests were similar to those previously reported, but, the time of the inoculations was changed. As far as the ordinary garden plants, hosts of crown gall, are concerned, it has

<sup>1</sup> Completed with a grant-in-aid made by the Chemical Foundation, N. Y.

been a generally established custom to inoculate them while still actively growing and young; that is, in the early part of the growing season, usually May, June, and early July in the North. In these experiments the date of the inoculation was set for the latter part of July and August up to early September. The joints studied in these experiments were grown in pots sunk in the ground in the experimental garden at Montefiore Hospital.

A very virulent strain of *Bacterium tumefaciens* known in my culture as 6NIS<sup>6</sup> was isolated a number of years ago from a nonsterilized fragment of geranium crown gall, which consisted of an interior and a surface portion of the neoplasm. The inoculations were made in the usual manner by piercing the edge of the joint with a sterile needle immersed in a culture of the organism.

The plants were removed from the garden the latter part of September and kept in a cool room where the temperature never exceeded 20° C. during the winter months, with diffused light on sunny days only. In January of the present year small swellings appeared on the edges of *Opuntia Keyensis*. In June these plants were set out in the garden again, when it was noticed that well-developed crown galls had been formed on a number of inoculated joints of this plant. Portions of these galls were fixed in Bouin's and Flemming's weak solutions. Paraffin sections 5  $\mu$  to 10  $\mu$  were stained in Heidenhain's iron haematoxylin and Flemming's triple stain. An excellent stain for this tissue consists of an aqueous solution of safranin counter-stained with light green dissolved in oil of cloves. The nuclei in these preparations are ruby red with nucleoli somewhat more deeply stained, while the cytoplasm is pale green and the cell walls become brilliant green. The other *Opuntias* inoculated have given no results other than development of necrotic areas at the region of bacterial implantation.

#### OBSERVATIONS

Inoculations of several species of *Opuntia* listed above with a virulent strain of *Bacterium tumefaciens* resulted only in the development of crown galls on the well-rooted joints of *O. Keyensis*. These galls begin to appear 4 to 5 months after inoculation and attain the size of hazelnuts in 6 to 7 months. Figure 1 shows 2 plants approximately a year after inoculation. The galls have grown to the size of a walnut and are smooth and sessile. They are pale greenish yellow with minute brownish to reddish spots on their surface. The galls grow slowly at first, but, like young joints once formed, make very rapid progress in development. At present (July, 1934) they have attained the size of well-formed oranges with no apparent necrosis. The joints of this species of *Opuntia* are characterized by weak spines and a closer examination of the surface of the gall reveals the presence of reddish brown papillae or soft spine or root-like structures. On

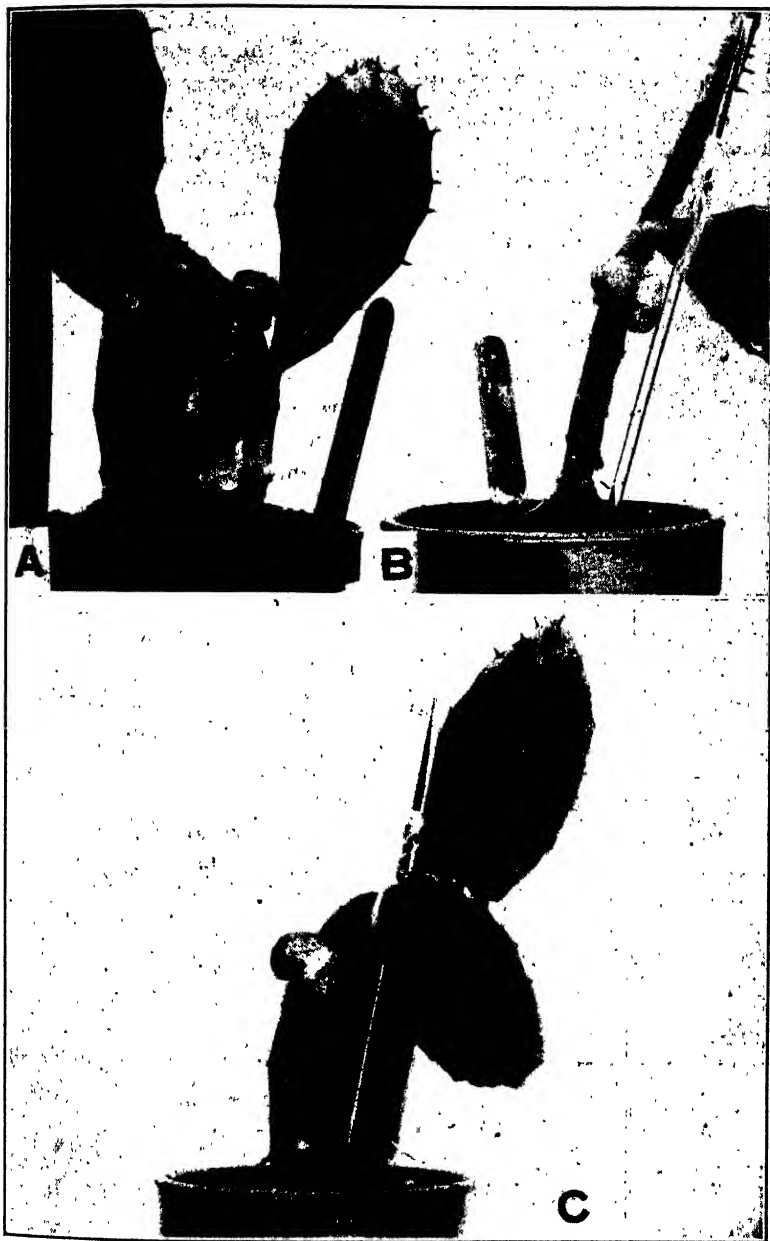


FIG. 1. *Oputia Keyensis*.  $\times \frac{1}{4}$ . A. Side view of joint with crown gall on edge of main shoot at the site of inoculation with *Bacterium tumefaciens* on Aug. 4, 1932. Photographed July 27, 1933. B and C. Another plant of the same species showing crown gall. Inoculated on Aug. 4, 1932, and photographed approximately one year later. Microscopic preparations were made principally from plant shown in A.

removing parts of these galls a bark-like coating is formed over the cut surface without any disturbance to the rest of the tumor. These galls have been watched carefully and, at present writing, no mature spines have been observed on the galls, as in the case of *Carnegiea gigantea*.

Microscopic sections of these tumors prove to be of great interest. The crown-gall tissue, as in the Sahuaro, is soft and sections very readily. A



FIG. 2. Section of crown gall showing conglomeration of disoriented parenchymatous and fibrovascular elements.  $\times 50$ .

photomicrograph of the histological structure of a gall under low magnification is shown in figure 2. Here one recognizes the compact corky structure of the surface consisting of several layers of cells in thickness, which is broken and peeling off; lying below and making up the body of the tumor, is a conglomeration of cells of various sizes. Cross and longitudinal sections of disoriented fibrovascular elements are seen surrounded by parenchymatous tissue. Scattered along the surface of the tumor mass, as

shown in figure 3, are a number of definitely organized apparently normal embryonic structures. In young stages these bodies appear to be globular (Fig. 3) and elongate as they grow older (Fig. 4). These structures in their early stages appear normal, consisting of a growing area made up of small embryonic cells that stain deeply, like the embryonic root tips of other plants. The nuclei are comparatively large, well differentiated, and division



FIG. 3. Section near surface of gall showing highly organized structures imbedded in the crown-gall tissue. Apparently it was developing roots. Note growing point made up of densely staining embryonic cells apparently growing through the cork-like surface of the gall.  $\times 50$ .

stages are numerous. This growing point lies near the periphery of the tumor, under the epidermis through which it appears to be growing. A middle or stalk portion made of larger cells appears to constitute the region of elongation, while the basal part of this stalk region, farthest away from the surface of the gall, consist of 2 or 3 layers of cells forming a definite wall about this structure. Here the cells are larger and the nuclei are well

differentiated and occupy a central position in the cytoplasm. This tissue shows a moderate number of cell and nuclear division stages. Cross-sections of these embryonic structures below the growing region appear frequently in my preparations (Fig. 5). Surrounding these embryonic structures are large cortical cells that are frequently binucleate. Many cells in this area are filled with aggregates of crystals.



FIG. 4. Abortive young root, necrotised, on surface of crown gall.  $\times 50$ .

In microscopic sections of some galls I have found aborted multicellular projections that are spine-like and probably represent the later development of roots described above. These structures (Fig. 4) arise in the parenchymatous tissue of the gall. Fresh and active fibrovascular elements appear to grow into these projections, while the epidermis and cortex

are dry and apparently dead. The tissue surrounding this abortive root consists of cortical cells; cells filled with aggregates of crystals are scattered through the tissue.

Clusters of embryonic cells and imperfectly organized embryonic roots also appear in the mass of parenchymatous tissue that constitutes the major part of the gall. Some of the former cells appear in the interior of the gall and ultimately give rise, by differentiation, to strands of fibrovascular elements. The latter appear near the periphery of the tumor under the surface of fragmentary masses of epidermal and cork cells. Their ultimate

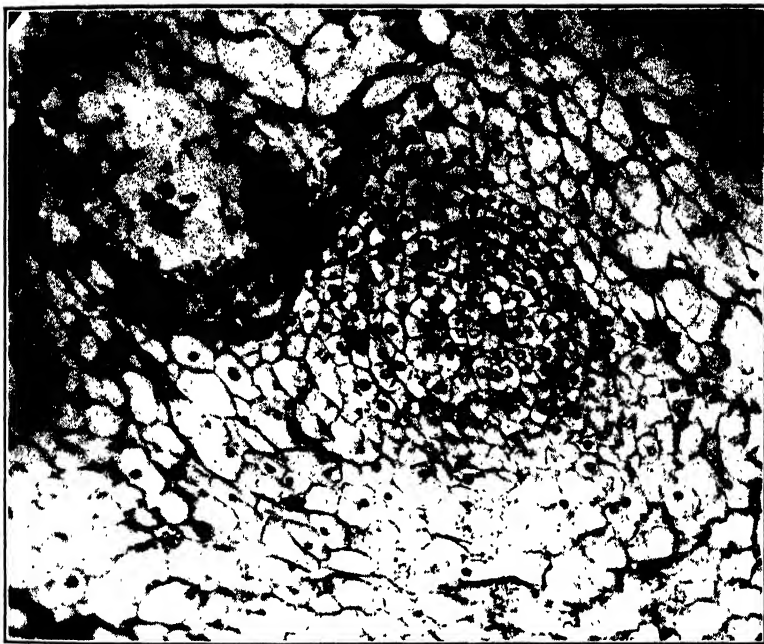


FIG. 5. Cross-section of an embryonic root imbedded in the crown gall shown in figure 3; well-differentiated nuclei are present.  $\times 100$ .

fate has not been followed, but it is likely they break down early in the aging of the gall. The parenchymatous or cortical cells became large and stained beautifully with the Flemming's triple stain. Binucleate cells appear in large numbers in the tissue under the epidermis studied.

It appears that the tumors of the *Opuntia* are not simple growths as shown in the small nodular neoplasia described for the beet (2) and Sahuaro but represent growths with a number of growing centers in the interior of the gall. The activity of these centers brings the old differentiated tissue of the gall to the surface. Growth in these tumors appears to be internal and peripheral.

Preparations for microscopic study were made of these plants so that the host and crown-gall tissue appear in the same section. Such a preparation is represented by figure 6. The small parenchymatous cells, to the left of the slightly elongated cells, represent the crown-gall tissue, while to the right is the host tissue with larger cortical cells. The large cells filled with stratified layers of mucilage or slime, similar to those described for the tree cactus (3), are found only in the host tissue. Zones of deeply stained material appear to surround these cells. The small black bodies in both tissues are cells filled with aggregates of crystals.

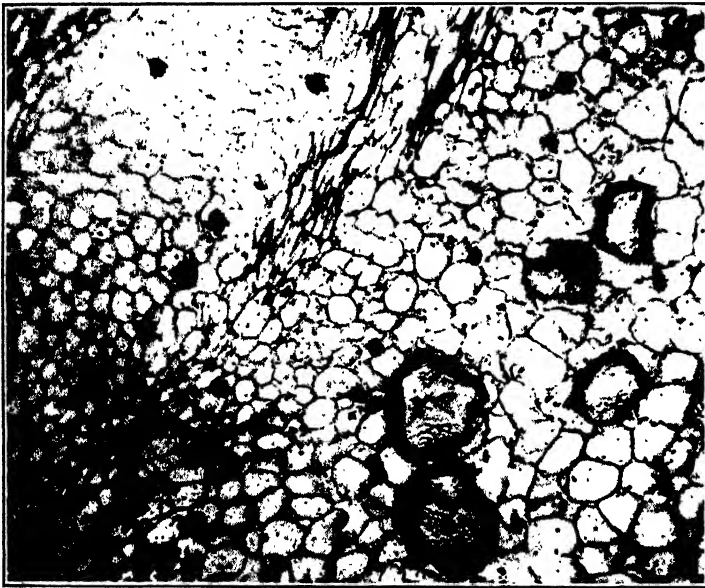


FIG. 6. Section through the host and crown-gall tissue separated by a strand of elongated parenchymatous cells. Note absence of mucilage or slime cells in crown-gall tissue on left of strand. Black bodies in both tissues are cells containing aggregates of crystals.  $\times 100$ .

#### SUMMARY

Crown gall on *Opuntia Keyensis* has been induced by inoculation with a virulent strain of *Bacterium tumefaciens*. Under conditions not yet clear *O. Keyensis* inoculated in the late summer and kept at moderately low temperatures produces in one year a crown gall the size of a black walnut. Microscopic studies of this gall show a disoriented conglomeration of cells consisting of fibrovascular elements, clusters of embryonic cells, and parenchymatous tissue surrounded by fragments of epidermis and bark. There are also a number of definitely organized structures that, to all appearances, are embryonic roots developing in the periphery of the crown gall. Their



cellular organization, it appears, is normal; although their further development results in structures, probably aborted roots. Many host as well as crown-gall cells show an aggregate of crystals, while only the host tissue contains mucilage or slime cells. The majority of *Opuntias* so far studied appear to lack the protecting mechanism of forming overgrowths common to a large number of the dicotyledonous plants in that the introduction of the tumor-producing organism results in injury and death of the tissue.

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# THE ROOT-KNOT NEMATODE ON RICE<sup>1</sup>

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(Accepted for publication November 22, 1933)

## INTRODUCTION

On June 4, 1932, some rice plants (*Oryza sativa* L.) were received from the farm of H. D. Seebre, about a mile south of the Rice Branch Experiment Station, Stuttgart, Arkansas. The specimens were accompanied by the information that on about 1½ acres on the upper side of the field the plants seemed to be dwarfed and were yellower and less vigorous than those in the rest of the field. No diagnosis was made from the plants received, as no evidence of disease was found.

Mr. Seebre's field was visited on June 8 and, on examination, it was found that the roots of many of the plants in the area referred to were deformed, as are the roots of plants infested with the root-knot nematode (*Heterodera marioni* (Cornu) Goodey).<sup>2</sup> Subsequently, this identification was verified by Dr. G. Steiner and Miss Edna M. Buhner, Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture. Some of the deformed roots were examined in the laboratory with the aid of a dissecting microscope, and 1 to 3 nematodes were found in each of them (Fig. 1). In a few cases young female stages of the parasite were found in the same stage of development, as shown in figure 2, A and B.

## INOCULATION EXPERIMENTS

The roots of some tobacco plants in the greenhouse were found to be parasitized by the root-knot nematode. These plants were lifted and the roots and the soil in which they had been growing were used for infection studies on plants of Supreme Blue Rose rice.

In the first experiment the rice seed was sown in the soil in the flat in which the tobacco plants had been growing. In the second experiment the root systems of the tobacco plants were used as inoculum. Eight 3-gallon stoneware jars were half filled with Clarksville silt loam and the tobacco roots were placed on the surface and covered with about an inch of soil.

<sup>1</sup> Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas Agricultural Experiment Station.

<sup>2</sup> According to Buhner, Cooper, and Steiner (Plant Disease Reporter 17: 64-96. June 15, 1933), *Heterodera marioni* (Cornu 1879) Goodey 1932 is the proper designation for the root-knot nematode. The names *Heterodera radiculicola* (Greeff 1872) Müller 1884, *Meloidogyne exigua* Goeldi 1887, *Anguillula arenaria* Neal 1889, *Tylenchus arenarius* Cobb 1901, and *Caconema radiculicola* Cobb 1924 are considered as synonyms.

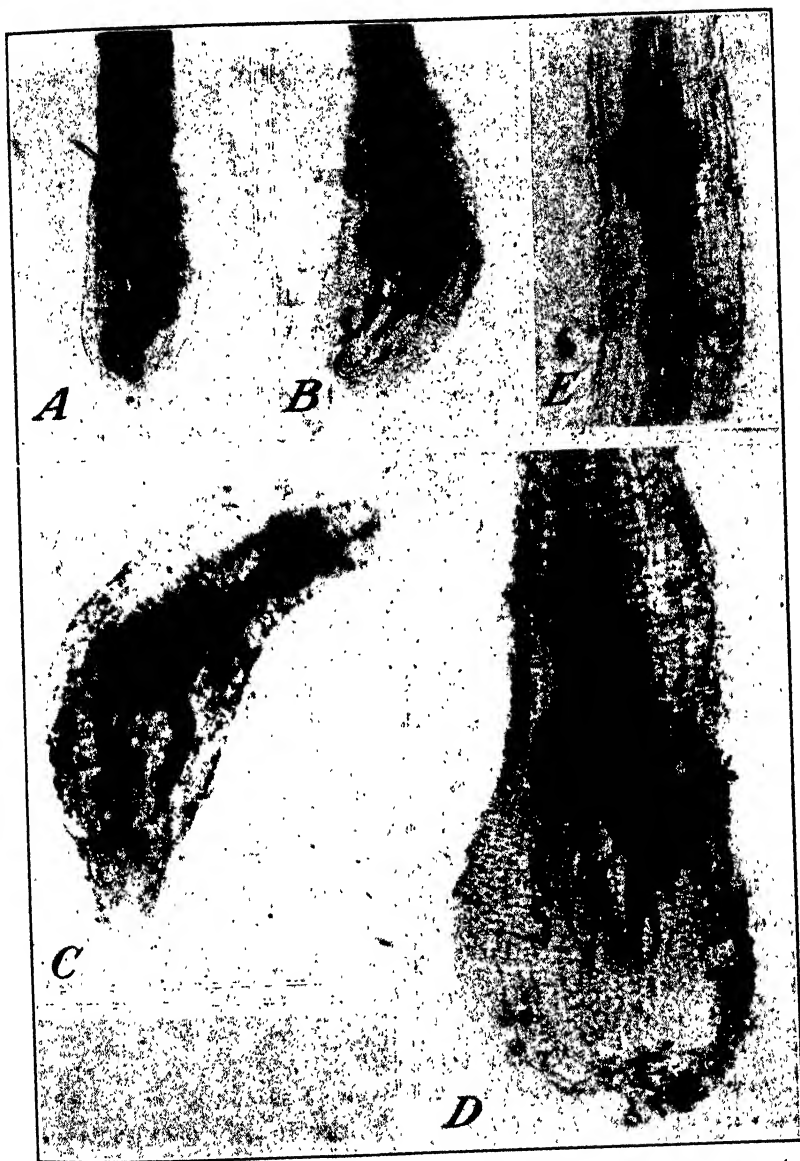


FIG. 1. *Heterodera marioni* in roots of Supreme Blue Rose rice plants. A-D. In tips of roots. E. At some distance back of tip.

Rice seed was then sown in the jars. At the end of 10 days the plants were 5 and 6 inches high. Enough water was added to the jars to maintain a constant half-inch depth of submergence. As a check against these inoculations, rice was sown in a jar of Clarksville silt-loam soil without tobacco

roots. The plants in this jar received the same treatment as the plants in the other jars. At the end of the first week some of the plants were removed from the flat and from one of the tobacco-nematode-infested jars. Typical root knot was found on these plants. The root systems of plants from infested jars are shown in figures 2, *C*, and 3, *A*. The roots shown in figure 3, *B*, are from the noninfested control jar.



FIG. 2. *A* and *B*. Early female stages of *Heterodera marioni* in roots of Supreme Blue Rose rice. *C*. *H. marioni* infested root system of Supreme Blue Rose rice plant, 10 days old, from jar inoculated with the nematodes from infested tobacco roots.  $\times 2$ .

Two plants in one of the jars were found to have abnormally developed tissues in the region of the subcoronal internode, as shown in figure 3, *A*. Nematodes were found in the sheath and crown tissues of both plants. According to Dr. Steiner, they were migrating larval specimens of the nematode *Heterodera marioni*. Dr. Steiner states that "in one swelling some 39 specimens were counted, none of which had become fixed and therefore no giant cells had been formed. That these tissues in the region of the subcoronal internode should be induced to swell by the simple presence of these migrating larvae seems very interesting and shows an interrelation of host and parasite not previously observed."

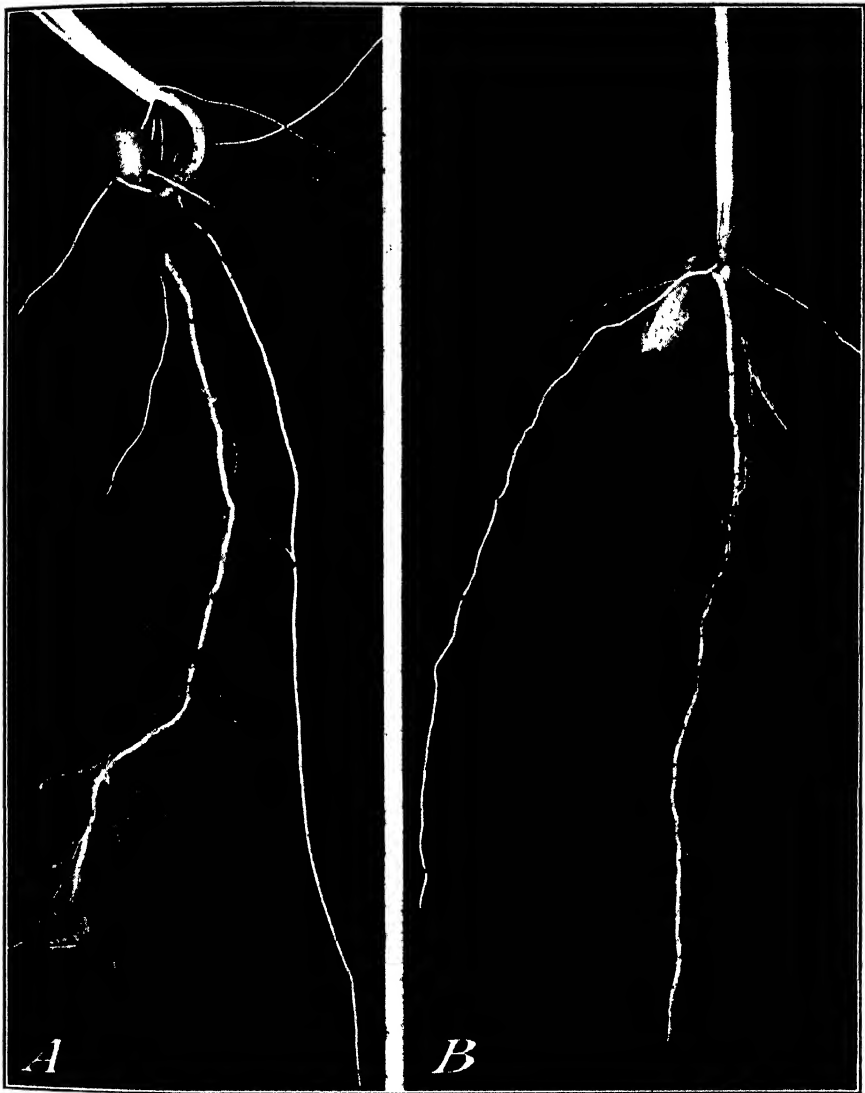


FIG. 3. *A.* Same as figure 2, *C*, except that the plant was 3 weeks old. Migrating larvae of *Heterodera marioni* were found in the tissues of the subcoronal internode and the coleoptile.  $\times 2$ . *B.* Root system of healthy Supreme Blue Rose rice plant, 3 weeks old, from control jar.  $\times 2$ .

The root systems of nearly all the plants grown in infested soil were parasitized. Marked reduction in root growth was noted; the roots of the parasitized plants had attained only half the length of those of the control plants. It also was found that a smaller number of roots was produced by

plants grown on infested soil than by the control plants. It is of considerable interest to note that the roots of plants grown under submerged conditions were more severely affected and contained larger numbers of nematodes than the roots of plants grown in the nonsubmerged flat. It is likely that the nematodes were attracted to the submerged rice roots because there probably was a greater supply of oxygen in the roots than in the soil. The aerial parts of infested plants were dwarfed, the average height being only half that of the controls, and many of them had been so weakened at the end of 3 weeks that they could not have survived much longer; therefore, readings were taken on all the plants at this time. The results are given in table 1.

TABLE 1.—*Infestation of roots of Supreme Blue Rose rice grown in soil infested with Heterodera marioni*

Grown in—	Number of plants with			Plants parasitized
	Total	Roots		
		Healthy	Parasitized	
				<i>Per cent</i>
Flat with infested soil . . . . .	169	5	164	96
Jar with infested tobacco roots	23	0	23	100
“	43	8	35	81
“	21	2	19	90
“	26	1	25	96
“	46	2	44	95
“	26	0	26	100
“	33	0	33	100
“	35	0	35	100
Control . . . . .	45	45	0	0

It will be seen from the data that Supreme Blue Rose rice roots were readily parasitized by the root-knot nematode under the conditions of the experiments. An average of 95 per cent of the roots of a total of 422 plants was parasitized. These infected roots were referred to Dr. G. Steiner, Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture, for special study. The results of his study and a review of the literature are given by him in the following article.

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## PHYTOPATHOLOGICAL NOTES

*Probable Occurrence of Australian Spotted Wilt of Tomatoes in Wisconsin.*—During the summer of 1930 a peculiar form of streak occurred on Globe tomatoes grown in the field at Madison, Wisconsin. The seedling plants were grown in the greenhouses of the Department of Plant Pathology of the University of Wisconsin and the primary infection apparently occurred before transplanting to the field. The disease was first noted when the plants were about 8 inches tall, the early symptoms consisting of a slight downward curling of the youngest leaves (Fig. 1, B). These leaves soon developed a yellowish green, bronze color, which in some cases occurred evenly along the fine veinlets and in other took the form of irregular or circular spots (Fig. 1, D and E). Within 3 to 4 days the bronze areas became dark brown and the affected leaflets withered and died. Elongated, bronze areas also developed on the petioles and upper portions of the stem. The tips of the plants usually withered and died within 10 to 14 days after the first symptoms appeared. The dead leaves were very brittle and of an extremely dark color (Fig. 1, A). The disease appeared in July and nearly all the plants infected at that time died. A few cases continued to develop during the rest of the summer but the injury was less severe as the plants became larger. In late August a few fruits were found that were strikingly marked with broad, concentric bands of light and dark green. As they matured the markings changed to concentric rings of orange yellow and red (Fig. 1, C). The source of the primary infection was not determined, but it was significant that the tomatoes had been held for a time in a cold frame that contained a number of ornamental plants.

The disease was readily transmitted by artificial inoculation with the expressed juices of bronzed plants, the inoculations being made by rubbing the leaflets with a cotton-tipped needle soaked in the inoculum and by inserting a bit of the cotton in a needle puncture made at one of the upper leaf axils of the stem. During July infection occurred in 50 per cent of the plants inoculated, but, as the greenhouse temperatures increased, the percentage of infection declined. The infected plants developed typical symptoms of the disease as seen in the field. Owing to circumstances, it was impossible to study the disease intensively during the summer of 1930 and little was done on the properties or host range of the virus. Attempts at thermal death-point determinations gave irregular and uncertain results, but the indications were that the thermal death point was in the neighborhood of 60° C. The virus did not live longer than 48 hours *in vitro*, but no shorter determinations were made. One series of Broadleaf tobacco plants developed an irregular necrosis and some distortion of the leaves, but no other host-range studies were made.



FIG. 1. A. Tip of young tomato plant showing withering of bronzed leaves and mild stem and petiole lesions. B. Tomato plant showing downward curling and bronzing of leaves at growing tip. C. Fruit of Globe tomato showing concentric ring markings. D. Tomato leaf showing various patterns of bronzed areas and withering of young leaflets. E. Leaf showing dark, circular spotting just preceding collapse of the leaflets.



On September 1, 1930, the senior writer transferred headquarters to Washington, D. C., and a stock of diseased plants was held for the time at Madison. The abnormally hot weather of September, however, deterred infection and all of the diseased plants died before further infection was obtained. As a result the studies were abandoned for the time.

The paper of Samuel, Bald, and Pittman<sup>1</sup> on spotted wilt of tomatoes in Australia was received at this time and the striking similarity of the two diseases was at once apparent. The behavior of the disease in Wisconsin was almost identical with their descriptions, and if the paper had been earlier available the plates on leaf symptoms could have been duplicated in almost all details. The possible identity of the two diseases was emphasized by the fact that Beecher and Shapovalov<sup>2</sup> had noted a similarity in the symptoms of the Australian disease and those of the die-back streak found in California. An abstract, therefore, was published by the writers<sup>3</sup> in 1931 in which the similarity of the Wisconsin disease to Australian spotted wilt was pointed out. Further publication was delayed in the hope that the disease might again occur at Madison; but, although tomatoes were grown on the same plots in 1931, it did not appear nor could it be found in the vicinity in 1933. Since the virus has not again been secured and since both Shapovalov<sup>4</sup> and McWhorter<sup>5</sup> are now working with viruses closely allied to or identical with the Australian or English forms of spotted wilt, it has seemed advisable to present the evidence on which the above abstract was based.

On the basis of symptoms there is no doubt that the Wisconsin streak apparently was identical with spotted wilt as described in Australia. The results of the thermal death-point studies, though inconclusive, indicate that the thermal death point of our virus may have differed somewhat from that of the Australian form as reported by Bald and Samuel<sup>6</sup> in a later paper. Since the Wisconsin studies were terminated in 1930, it is not possible to say definitely that the virus in question was identical with that oc-

<sup>1</sup> Samuel, G., J. G. Bald, and H. A. Pittman. Investigations on "spotted wilt" of tomatoes. Coun. Sci. & Indus. Res. Australia. Bull. 44. 1930.

<sup>2</sup> Beecher, F. S., and M. Shapovalov. Tomato die-back or tip blight found in inland areas of California. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 13: 148. 1929. (Mimeographed.)

<sup>3</sup> Doolittle, S. P., and C. B. Sumner. The occurrence of the Australian spotted wilt of tomatoes in Wisconsin. (Abst.) Phytopath. 21: 106. 1931.

<sup>4</sup> Shapovalov, M. The die-back form of tomato streak. (Abst.) Phytopath. 23: 928. 1933.

<sup>5</sup> McWhorter, Frank P. English form of tomato spotted wilt found in Oregon greenhouse. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Repr. 18: 25-26. 1934. (Mimeographed.)

<sup>6</sup> Bald, J. G., and G. Samuel. Investigations on "spotted wilt" of tomatoes.—II. Coun. Sci. & Indus. Res. Australia. Bull. 54. 1931.

curring in Australia, although the writers believe that this probably was the case.—S. P. DOOLITTLE, Senior Pathologist, and C. B. SUMMER, formerly Agent, Bureau of Plant Industry, United States Department of Agriculture.

*Preliminary Note on another Foot Rot of Wheat and Oats in Oregon.*—

For the past four seasons a foot rot of winter wheat and winter oats has been under observation by the writer in western Oregon. The disease occurs locally on certain red sandstone-shale soils, acid in reaction, in the humid coastal portions of Lincoln and Lane counties. The symptoms of the disease resemble closely those caused by *Gibellina cerealis* Passerini on winter wheat in the humid portions of northern Italy and of Hungary.<sup>1 2 3</sup>

The disease in Oregon is characterized by elongated, pale, dark bordered lesions in the lower leaf sheaths and basal nodes of the culms (fig. 1, A, B, C, D). In early May, a dirty-white mat of mycelium is formed on the lesions on the outer sheaths and between the underlying sheaths. This mycelial mat gradually becomes more compact and forms a velvety, white felt or stroma in which are imbedded small, yellow to brown abortive fruiting bodies. In a few cases immature perithecia, presumably of the same fungus, have been found in material collected in Lincoln County. No mature perithecia have been found during the four years the disease has been under observation. The immature perithecia resemble closely perithecia of *Gibellina cerealis*. According to Ferraris,<sup>3</sup> perithecia of *G. cerealis* require a year to mature. It is thought possibly that the dry summers in Oregon are not favorable for maturing the perithecia and that this may explain why mature perithecia have not been found.

From the lesions on wheat and oats the fungus was readily isolated on potato-dextrose agar on which it produced a rapid-growing, loose to floccose, dirty-white mycelium (fig. 1, E). After the initial loose growth of mycelium was produced, sclerotium-like bodies were formed. These were pale yellow at first, and later became bright orange and finally brown. At first they were "cheesy" and later became rather firm and rubbery, but scarcely carbonaceous. They were hemispherical and measured up to 6 mm in diameter. Sometimes they aggregated to form masses of considerable size.

Through the courtesy of Dr. G. Savastano and Director L. Petri and coworkers of the Royal Station of Vegetable Pathology at Rome, two new isolations of *Gibellina cerealis* were obtained from the interior of Trent in

<sup>1</sup> Passerini, G. Un' altra nebbia del frumento. Bol. Comizio Agr. Parm. No. 7. 1886.

<sup>2</sup> Anon. Une nouvelle maladie du froment. Rev. Mycol. 8: 177-178, Oct. 1, 1886.

<sup>3</sup> Ferraris, T. Mal bianco degli steli di grano [White disease of wheat stems]. Revista Agricola 26: 407-408. 1930.

northern Italy. These cultures (fig. 1, F) were identical with those isolated from the diseased wheat and oats collected in western Oregon. Therefore, the fungus that occurs on wheat and oats in western Oregon is tentatively designated as *Gibellina cerealis* Pass.

In pure culture, *Gibellina cerealis* from Oregon grows slightly at 6° C., slowly at 12° C., well from 18° to 24° C., and slightly at 27° C., and ceases to grow at 30° C.

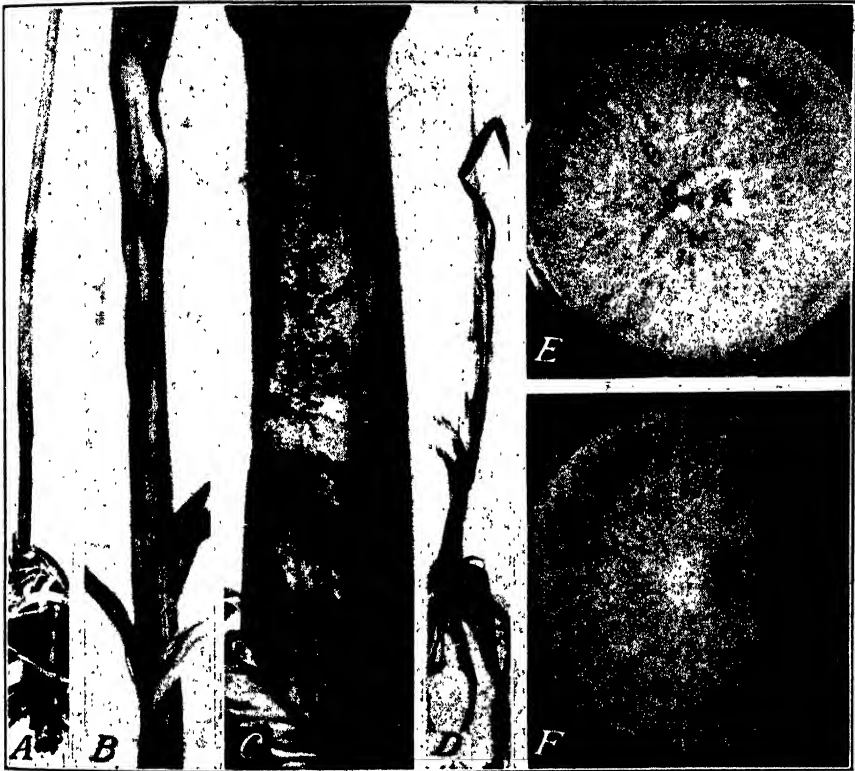


FIG. 1. *Gibellina cerealis* on wheat, barley, and oats and in pure culture. A. Lesions on Gold Coin wheat,  $\times 5/6$ . B. Lesions on Utah Winter barley,  $\times 5/6$ . C. Lesion on culm of Utah Winter barley enlarged to show developing stromata,  $\times 4$ , all following artificial pure-culture inoculations made in greenhouse, Corvallis, Oreg., October, 1932, and specimens photographed March 10, 1933. D. Lesions on Winter Turf oats collected in the field, Alsea River Valley, June, 1930,  $\times 1$ . E. and F. 14-day-old cultures of *Gibellina cerealis* on potato-dextrose agar from Oregon and Italy, respectively.

In artificial inoculations in the greenhouse the fungus was very pathogenic to winter oats, winter wheat, and winter barley when introduced with the seed. In this series of inoculations, the fungus caused a destructive seedling blight. The seedlings that survived fell prey later to a character-

istic attack when they were at a stage corresponding with that of early May in the field (fig. 1, A, B, C).

During the season of 1933 there was considerable breaking-over in winter wheat in the Gibellina foot-rot nursery in the Alsea River Valley of Oregon. The culms broke over at the lesions in a manner similar to the breaking-over caused by *Cercospora herpotrichoides* Fron.<sup>4</sup> However, the two diseases are readily distinguished by the characteristics of the stromata produced by the respective fungi. The stromata produced by *Gibellina cerealis* are spongy and almost white, whereas those produced by *C. herpotrichoides* are black and carbonaceous. Furthermore, in Oregon there is no danger, at present, of confusing these diseases as the *Cercospora* foot rot occurs east of the Cascade Mountains in an entirely different vegetational area,<sup>5</sup> whereas the Gibellina foot rot is known only in certain humid coastal regions of western Oregon.

In the field, barley will not grow on the acid soil infested with *Gibellina cerealis*. Soft, red winter wheats will grow on such soils, but most other wheats will not. All varieties of wheat thus far tried have been susceptible to the fungus. Winter oats resist the acid-soil condition and are only moderately susceptible to the fungus. Both wheat and oats that grow on the acid soil of coastal Oregon are attacked by a number of soil-borne organisms associated with *G. cerealis*. Oats are attacked also by *Helminthosporium avenae* Eidam, which causes at least part of the foot rot briefly mentioned in an earlier article.<sup>6</sup>

The most desirable cereals to grow for feed on the acid soils infested with *Gibellina cerealis* and other soil-borne fungi are Winter Turf oats and Alstroum spelt, which latter is resistant to the Gibellina disease.—RODERICK SPRAGUE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, cooperating with the Oregon State Agricultural Experiment Station, Corvallis, Oreg.

*Weather Conditions Associated with Seasons of Severe and Slight Celery Early-Blight Epidemics in Florida.*—Celery is grown around Sanford, Florida, in a very intensive manner. It may be planted as early as June and harvest may extend to the latter part of May. The crop, after 6 to 12 weeks in the seed beds, is moved to the fields where it is cultivated for 5 to 8 months. In Sanford, early blight (*Cercospora apii* Fres.) is the most important fungus disease of celery. It is widespread and appears first

<sup>4</sup> Sprague, R., and Hurley Fellows. The cercospora foot rot of winter cereals. U. S. Dept. Agr. Tech. Bul. 428. [In press.]

<sup>5</sup> Sprague, R. The association of *Cercospora herpotrichoides* Fron. with the *Festuca* consociation. *Phytopath.* 24: 669-676. 1934.

<sup>6</sup> Sprague, R. The distribution of cereal foot rots in the Pacific Northwest. *Northwest Science* 5: 10-12. \* 1931.

on the young seedlings. It is combated by a Bordeaux spray used in the seed beds and fields continuously, at 4- to 10-day intervals, until harvest time. Under ordinary conditions there the spray is applied at about weekly intervals. This varies, however, with weather conditions, and when fogs and heavy dews occur spraying may be done for considerable periods of time once and often twice a day.

The writer reported<sup>1</sup> a severe outbreak of early blight of celery in Florida during the winter of 1931-32. This was ascribed to "rainless periods accompanied by gentle eastern trade winds, in which the days are bright and warm, the nights slightly cool, the dews heavy and not drying off the plants until nearly noon." This type of weather and its effect on disease occurring were discussed a little more in detail in a later report.<sup>2</sup>

The winter growing seasons of 1931-32 and 1933-34 were markedly different from the standpoint of the amount of early-blight infection. In 1931-32 a severe epidemic occurred. Well over twice as much Bordeaux was applied as ordinarily; and, nevertheless, the blight even then was not held in complete check. In 1933-34 early blight was not serious; about a quarter of the amount of spray employed in 1931-32 was used and was found to keep the fields, commercially speaking, blight-free.

Temperatures have been implicated by many writers, but most carefully studied and discussed by Klotz,<sup>3</sup> as important in an epidemic of celery early blight. Judging from data given by Klotz, minimum temperatures for fungous activity are below 40° F. He found favorable temperatures for spore production to be 60° to 69° and for germination, 51° to 97°; for mycelial growth, 71° to 86°. He also found that spores are wind-blown, germinate on leaf surfaces in the presence of drops of moisture, such as are furnished by dew and fog, and infect leaves through the stomata in from 2 to 5 hours, regardless of light conditions.

The 5 critical winter months in Sanford, so far as celery early blight is concerned, are November, December, January, February, and March. According to United States Weather Bureau reports, high temperatures for these months in 1931-32 were between 85° and 89° F. and low temperatures were not much below 45° and only occasionally less than that for short periods. The monthly mean averaged 69°, which was 5.8° higher than the expected normal. In 1933-34 high temperatures were between 81° and 86°, and low temperatures were around 40° and occasionally slightly below. The mean for the season was just about the expected normal.

<sup>1</sup> Wellman, F. L. An unusual outbreak of celery early-blight. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 16: 43-45. 1932.

<sup>2</sup> ———. Pathological effects of the east wind in Florida. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. 17: 177-181. 1933.

<sup>3</sup> Klotz, I. J. A study of the early-blight fungus, *Cercospora apii* Fres. Mich. Agr. Exp. Sta. Tech. Bull. 63. 1923.

Under ordinary Florida winter conditions, temperatures, on the whole, are favorable to the development of *Cercospora apii* and around Sanford, at least, this alone could hardly be the limiting factor in early-blight epidemic. During the months studied in 1931-32 the daily precipitation of .01 inch or more on 28 days amounted to a total of 7.18 inches, and a severe epidemic of early blight occurred. Over the same period in 1933-34 it rained on 29 days a total of 13.25 inches, and the blight was of unusually small consequence. Thus, temperature and rainfall do not appear to be responsible for the great variation in severity of blight in these two seasons.

From the standpoint of celery early blight, the most extreme variable around Sanford seems to have been the relative amounts of fog and dew. During the winter of 1931-32 frequent warm east winds off the Atlantic Ocean brought in or induced fogs and much dew, and at the same time heavy *Cercospora* infection of celery resulted. In contradistinction to this was the light infection of *Cercospora* during the winter of 1933-34, a season marked by the regular occurrence of cool northerly dry winds that served to keep the Sanford region comparatively free from fogs and dew.—F. L. WELLMAN, Associate Pathologist, Bureau of Plant Industry, Washington, D. C.

*A New Wilt of Peas.*—There have been isolated repeatedly from the vascular bundles of the root and epicotyl of peas two or three species of *Fusaria* that differ specifically and pathogenically from the pea wilt organism, *Fusarium orthoceras* App. & Wr. var. *pisi* Linford. The wilt organism causes symptoms that have been described by other investigators in detail. It attacks only certain varieties, and from some of the susceptible varieties resistant strains have been developed by breeding and selection.

Many plants of wilt-resistant varieties and strains as well as susceptible varieties often display symptoms that are not characteristic of the common wilt. While growth is somewhat retarded the plants usually attain almost normal size and bear some pods. The leaves turn yellow, beginning with the lowermost ones which are followed consecutively by the next above. In the later stages they become dry and papery. They may curl downward and inward in a manner characteristic of the common wilt.

Either the vascular bundles of the hypocotyl or epicotyl or both may become a brick-red to a bright red color. The discoloration may involve most of the root, but usually it is not found far up in the stem.

This new wilt is widely distributed. The organisms have been isolated from plants collected or sent in from Maryland, Ohio, Virginia, South Carolina, Wisconsin, Colorado, Washington and Idaho. It probably can be found wherever peas are grown.

From the discolored vascular bundles of diseased plants, two species of

*Fusarium* have been isolated which are decidedly different from *Fusarium orthoceros* var. *pisi*, the cause of the common pea wilt. One species has been identified<sup>1</sup> as *F. oxysporum* Schl. var. *aurantiacum*<sup>2</sup> (Lk.) Wr. Another species somewhat similar but differing in certain cultural and morphological characters is found less frequently and has been identified provisionally as *F. vasinfectum* Atk. var. *lutulatum* (Sherb.) Wr. A third species, probably *F. redolens* Wr. was isolated in a few cases. This species was originally isolated by Wollenweber from peas and was claimed by him to cause<sup>3</sup> a wilt and foot disease of peas.

It has been possible to reproduce the disease by planting peas in sterilized soil profusely inoculated with cultures of both *F. oxysporum* var. *aurantiacum* and *F. vasinfectum* var. *lutulatum*. The first crop has yielded as high as 30 per cent infected plants. No inoculations were made with *F. redolens* but it is likely that this species, and probably others, will cause a similar disease. It is interesting to note that peas planted in virgin soils will yield the characteristic symptoms under greenhouse as well as under field conditions, showing that the causal organisms are general in their distribution.

The percentage of infected plants has been found higher in those soils repeatedly grown to peas.—L. L. HARTER, U. S. Department of Agriculture, Washington, D. C.

<sup>1</sup> Classified according to the system of Wollenweber in *Fusarium-Monographie. Fungi parasitici et saprophytici. Zeitschr. Parasitenk.* 3: 269–516. 1931.

<sup>2</sup> The writer wishes to acknowledge with thanks his indebtedness to Dr. C. D. Sherbakoff who examined and assisted in the identification of *F. oxysporum* var. *aurantiacum* and *F. vasinfectum* var. *lutulatum*.

<sup>3</sup> Wollenweber, H. W. Studies on the *Fusarium* problem. *Phytopath.* 3: 24–50. 1913.





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## PHYTOPATHOLOGICAL AND TAXONOMIC ASPECTS OF OPHIOBOLUS, PYRENOPHORA, HELMINTHOSPORIUM, AND A NEW GENUS, COCHLIOBOLUS

CHARLES DRECHSLER

(Accepted for publication December 13, 1933)

### OPHIOBOLUS HERPOTRICHUS AND O. GRAMINIS

The genus *Ophiobolus*, erected by Riess (65) in 1854, was extended into the sphere of phytopathological interest in 1881 with the transfer to it by Saccardo (66) of the fungus described by him (67) only 6 years earlier as *Rhaphidophora graminis* together with the form that, originally described by Fries (26, v. 2, p. 504) as *Sphaeria herpotricha*, later had been cited as *Rhaphidospora herpotricha* (Fries) by Fuckel (28), and as *Rhaphidophora herpotricha* by the Tulasne brothers (73). The transfer, as is evident from a statement of somewhat later date (68, v. 2, p. 337), was carried out because of nomenclatorial necessity, the genera *Rhaphidospora* Fries and *Rhaphidophora* Ces. and De Not., assuming they can be regarded as separate taxonomic entities on strictly formal grounds, having been found homonymous with genera of seed plants erected earlier by Nees and Hasskarl, respectively.

The earlier descriptive literature of the 2 fungi conveys little intimation of pathogenic relationships, the only direct intimation, indeed, here being contained in the somewhat reluctant statement by the Tulasne brothers that they had found *Ophiobolus herpotrichus* on fading wheat culms as early as the end of June, its appearance there being associated with subsequent premature yellowing of the heads and blackening of the stems. In 1880 Cugini (6) found a wheat disease in Italy, associated with blackening and killing of the roots, and with the development of a black incrustation over the surface of the stem. This disease he attributed to *Rhaphidospora herpotricha*, on the strength of an identification by Passerini; and the same fungus, referred to as *O. herpotrichus*, was held responsible for a similar disease encountered by Cugini (7) in 1889. In the meantime Morini (55), in investigating the causes of a new disease of wheat in Italy, had discovered, though apparently only on a single diseased specimen, a fungus that he held to agree well with *O. herpotrichus* except in having shorter asci and

ascospores, these structures measuring only 90–114  $\mu$  and 75–84  $\mu$ , respectively. This fungus he had described as *O. herpotrichus* var. *breviasci*.

The dimensions given by Morini together with his characterization of the ascospores as pale yellowish make it hard to avoid the conclusion that he was dealing not with *Ophiobolus herpotrichus* at all, but with *O. graminis*. Of the latter species he makes no mention, a fact that, under the circumstances, is difficult to explain except on the assumption that he was unaware of its having been described. For that matter neither of Cugini's papers makes mention of *O. graminis*, though the statement in the later one to the effect that Saccardo had said in the "Sylloge fungorum" that the species involved (presumably *O. herpotrichus*) was found on *Cynodon* and *Agrostis*, indicates that Cugini may have inadvertently confused or, perhaps, purposely integrated the portion of Saccardo's diagnosis of *O. graminis*, referring to habitat (in which *Cynodon* and *Agropyron* were cited as hosts) with the diagnosis of *O. herpotrichus*. It is possible therefore that Cugini's exclusive preoccupation with *O. herpotrichus* may have been due less to lack of knowledge concerning the other form reported on graminaceous hosts than to doubt as to the independence of that form from the much older and better established species the graminicolous character of which apparently had already become somewhat a matter of accepted tradition.

Such doubt, at all events, would seem to have prevailed after Prillieux and Delacroix (61) in 1890 reported *Ophiobolus graminis* as the cause of the wheat disease in France long known there as *piétin* or *maladie du pied*. For Frank (22), in reporting *O. herpotrichus* as responsible for serious damage to wheat in Germany in 1894, stated not only that the fungus had been known for some time in Italy, but also that it had a few years earlier been observed in the region surrounding Paris, there having occasioned the same injuries as in Germany. Any possible uncertainty as to which French report was alluded to in the statement, was dispelled in two later publications (23; 24, v. 2, p. 306–307) in the second of which, especially, the paper by Prillieux and Delacroix was explicitly cited in connection with the representation that *O. herpotrichus* had been found in France as the cause of *piétin* or *maladie du pied*. As the French authors had clearly set forth that their identification of the parasite causing *piétin* was based on its correspondence to Saccardo's description of *O. graminis*, especially with respect to dimensions of asci and ascospores, and as Frank was evidently cognizant of the different dimensions attributed to *O. herpotrichus*, since he cited them in one of his publications (24), it may be inferred that he regarded the 2 species as one in spite of knowledge of the differences in morphology ascribed to them.

This inclusive species concept became of moment in phytopathological

literature when in Frank's several publications *Ophiobolus herpotrichus* was set forth as one of the most destructive parasites of wheat in Germany, with the imputed destructiveness being, moreover, vividly brought into relief in the formidable term *Weizenhalmtöter* applied to the fungus. Unfortunately, these papers contain few clues as to the extent to which their author may have been dealing with *O. herpotrichus* on the one hand and with *O. graminis* on the other. The most tangible clue undoubtedly is to be found in the colored plate accompanying the last of Frank's (25) papers on the *Weizenhalmtöter*, which it may be presumed was prepared under his direction or must at least have met with his approval. In this plate the figures of the asci and ascospores show proportions assuredly much more suggestive of *O. graminis* than of *O. herpotrichus*. Later, to be sure, Krüger (41), on the basis of actual identifications, concluded that *O. herpotrichus*, evidently in its true restricted sense, was, in spite of unimpressive pathogenicity under experimental conditions, the species generally concerned in Germany, and he held this conclusion to confirm an assumption to the same effect that he credited to Frank. Whatever assumptions may be credited to Frank would, however, judging from his published works, need to be predicated on an *O. herpotrichus* including within its limits both the *O. herpotrichus* and the *O. graminis* of other authors. In contrast to Krüger, McAlpine evidently fully understood Frank's ambiguous application of *O. herpotrichus*. This author, who at first (45) considered the "wheat-stem-killer (*Ophiobolus herpotrichus*)" in relation to "take-all," which had long been destructive to wheat in Australia, later (46) referred the disease to *O. graminis*, explaining that the fungus in question was identical with the wheat-stem-killer determined by Frank in 1894, as well as with the parasite found by Prillieux and Delacroix to be the cause of footrot in France. It is not apparent that the ambiguity was recognized by van Hall (30), whose extended account of a disease of wheat in Holland, which he attributed to the *tarwehalmdooder*, added to the reputation of *O. herpotrichus* as a serious pathogen.

This reputation, however, during the 3 succeeding decades, has not been fully sustained, though Crüger (5), as late as 1929, attributed to *Ophiobolus herpotrichus* an important rôle in the causation of foot disease. Owing to the unimpressive performance of the fungus under ordinary experimental conditions, Foex and Rosella (20, 21) in France, van de Laar (43) in The Netherlands and Schaffnit (69) in Germany, have come to regard it as a parasite of secondary importance. Rather curiously *O. herpotrichus* has, hitherto, apparently never been recorded as occurring in the United States, though the writer has found a fungus morphologically agreeing with and evidently referable to that species regularly occurring in great abundance on quack grass (*Agropyron repens* (L.) Beauv.) during April, May,

and early June in Maryland, the District of Columbia, and Virginia every year from 1922 to 1933, as well as near Madison, Wis., in the corresponding seasonal periods in 1919 and 1920, and on Long Island in and about Brooklyn, N. Y., in 1921. Evidently quack grass serves, at least in the northern part of this country, as a most congenial host of *O. herpotrichus*, and but for the low esteem in which this grass is held the reputed stem-killer of central and western Europe might long have been a familiar object to American pathologists. If, as seems not unlikely, the fungus is similarly frequent in northern and central Europe, the earlier reluctance of investigators in those regions to accept *O. graminis* as a separate species has some explanation. For Saccardo's citation of *Agropyron* as one of the two genera representative of the hosts of *O. graminis* could hardly have failed to convey somewhat the impression that the *Ophiobolus* occurring so abundantly and so frequently to the exclusion of any congeneric fungus on the very widely distributed member of that host genus must somehow have been the one on which his diagnosis was based.

Although the paper of Mangin (50) published in 1899, cast doubt on the causal relationship of *Ophiobolus graminis* to *piétin* in France, Delacroix (10), 2 years later, reestablished the position of the fungus as at least one of the parasites involved in that disease. In 1901 appeared also a report by Hori (36) attributing foot rot in Japan to *O. graminis*, and in 1904 McAlpine's account, already referred to, connecting the fungus with take-all in Australia. Thereafter the destructively pathogenic character of *O. graminis* appears never to have been seriously questioned. In the United States the fungus and the disease caused by it have been treated more especially in publication by Kirby (39, 40), Davis (9), and McKinney (47); while recent European testimony to the harmful effectiveness of *O. graminis* is given by Foex and Rosella, by van de Laar, by Schaffnit, and by Moritz (56).

Such degree of confusion as earlier obtained with respect to *Ophiobolus graminis* and *O. herpotrichus* had, it may be admitted, some excuse in the resemblance in habit between the 2 fungi. Similarities such as those expressed in the rather characteristic localization of the perithecia on the basal portions of grass culms, and in the presence of the superficial network of dark hyphae over the adjacent host substratum, constitute features of parallelism that would seem to indicate a narrowly congeneric relationship. Since these features are set forth also in Tullis' (74) account of *O. oryzinus* Sacc., the cause of a rice disease in Arkansas, and are sustained here besides by general similarities in dimensions of asci, as well as in dimensions, septation, and arrangement of ascospores, there is reason to believe that this parasite, too, is included in the same intimate relationship. *O. cariceti* (B. and Br.) Sacc., described originally from *Aira caespitosa* in

England, was found by Fitzpatrick, Thomas, and Kirby (18) to resemble the take-all fungus so closely in morphology of perithecium, asci, and ascospores that they held it to be the same organism and, accordingly, favored relegation of the name *O. graminis* to synonymy. However, as the only material of *O. cariceti* used in their comparisons consisted of microscope preparations and herbarium specimens no longer in living condition and, moreover, not entirely above question in regard to authenticity, the resemblances observed by them have, in conformity with representations made by McKinney, been generally deemed insufficient to justify so important a nomenclatorial change as the one they proposed. Of course, the possibility of such identity, earlier predicated by Berlese (2, v. 2, p. 119-120) in his citation of both *O. cariceti* and *O. graminis* as synonyms of *O. eucryptus* (B. and Br.) Sacc., is not yet to be dismissed; and, in any case, the resemblances referred to together with the similarity in position on the host, also pointed out by Fitzpatrick, Thomas, and Kirby, would seem to indicate a close relationship in the same series.

It appears probable that *Ophiobolus oryzae* described by Miyake (54) from rice in Japan may likewise belong in the same group with *O. graminis* and *O. herpotrichus*, even though its occurrence on the leaves and glumes of the host plant rather than on the basal parts fails to sustain the parallelism in parasitic habit. As to various other species of *Ophiobolus* recorded from dead graminaceous materials, as, for example, *O. medusae* E. and E. on culms of *Spartina* sp. in New Jersey, *O. festucae* Tracy and Earle on dead leaves of *Festuca* sp. in Colorado, *O. trichosporus* Ell. and Ev. on grass stems in Canada, *O.* (later *Ophiochaeta*) *trichellus* Bomm. Rouss. Sacc. on the lower leaves of *Psamma arenaria* Roem. and Schult. in Belgium, *O. seriatus* Syd. on dead stems of *Bambusa* sp. in the Philippines, *O. graffianus* Sacc. on the decaying caryopsis of *Coix lacrima-Jobi* L. in the Philippines, and *O. panici* Syd. on dead sheaths of *Panicum miliaceum* in the Philippines, the available information is too scanty for any conjecture as to their more intimate affinities. In the main, no doubt, these species owe their assignment to *Ophiobolus* much more to gross conformity with the broad Saccardian application of the genus, than to their degree of similarity to the 2 parasites through which the genus has become familiar to plant pathologists.

#### HISTORICAL AND TAXONOMIC ASPECTS OF THE GENUS OPHIOBOLUS

##### IN GENERAL

For, although *Ophiobolus graminis* and *O. herpotrichus* have occupied prominent places in mycological literature, their influence on the taxonomic history of the genus in which they are now included has remained unimportant. Indeed, in their present systematic setting, there is little historical

justification for regarding either the one or the other as a pivotal species. *Ophiobolus* was erected on a fungus found presumably in saprophytic relationship on *Carduus arvensis* Robs., and described as new by Riess under the name *Ophiobolus disseminans*. The newness of the species, to be sure, failed to convince contemporaneous writers some of whom, like Duby (63, no. 57), evidently considered it to be identical with the fungus that Sowerby (71, v. 3, pl. 394, fig. 3) had much earlier found "scattered on the stalk of a thistle" and described rather inadequately as *Sphaeria acuminata*, while others held it to be the same as Wallroth's (77) *Sphaeria carduorum*, of which the host substratum was only slightly more definitely indicated in the words "*ad caules Carduorum siccos.*" Apart from the question of specific identity the fungus, according to Riess' description, was certainly not devoid of morphological distinctiveness, the many-septate ascospores being set forth as originating joined end to end in pairs, later to become separated individually at a commissure located between two swollen segments, and thus finally appear with the characteristic snakelike terminal swelling signalized in the generic name. Yet neither the rather astonishing development ascribed to the ascospores, which now would more likely be interpreted as representing disarticulation of a single ascospore into halves, nor the arresting nodosity of the inflated spore segments appears to have impressed writers of the time as constituting a character of generic significance. The disesteem in which both the genus and species launched by Riess soon came to be held is reflected in the use of the various binomials applied presumably to the same fungus, Rabenhorst (63, no. 530) citing it as *Rhaphidospora disseminans*, the Tulasne brothers as *Rhaphidophora carduorum* and Fuckel as *Rhaphidospora carduorum*.

In the same publication with the latter citation Fuckel further cited under *Rhaphidospora*, *R. rudis* (Riess) and *R. rubella* (P.), thereby in effect merging with *Rhaphidospora*, in addition to *Ophiobolus*, the 2 genera typified in these species. Of these 2 genera *Entodesmium* had been erected by Riess in 1854 on the newly described *E. rude*, the spores of which were set forth as being produced within clavate asci in chains of approximately 16 individuals,—a characterization now more aptly interpreted as referring to ascospores with approximately 15 septa disarticulating into their component segments. *Leptospora*, the second of the amalgamated genera, had been erected by Rabenhorst (62) in 1854 on a fungus he regarded as identical with the *Sphaeria porphyrogena* described as new by Tode (72) in 1791, but treated by Persoon (60) in 1801 as a variety of *Sphaeria rubella*. The distinctive feature setting his genus apart from *Rhaphidospora* was found, according to Rabenhorst, in the non-septate character of the ascospores, but as early as in 1863, Cesati and De Notaris (3), whether from doubt as to the reality or as to the significance of this imputed feature, cited the presumptive type species as *Rhaphidophora porphyrogena*.

In any case it is apparent that long before the publication of the *Sylloge fungorum* began, species of types sufficiently distinctive to have led earlier to the proposal of several separate genera had wisely or unwisely been brought into the same fold. Favored no doubt by the distinguished example set by the Tulasne brothers, the term *Rhaphidophora* apparently proposed by Cesati and De Notaris as a mere modification to avoid homonymy with Nees' genus, continued for about 2 decades to hold the field, though the earlier term reappeared now and then, as in Fuckel's meritorious work already cited. When it was finally realized that, curiously enough, the substituted term was a homonym no less than the one it displaced, Saccardo, as has been noted previously, revived *Ophiobolus*, applying it, of course, not only to the forms corresponding closely to the type on which it was erected, but also to the multitude of forms corresponding to the several other types with which its original type had in the meantime become agglutinated. In the second volume of the "*Sylloge fungorum*," although a number of species of the abandoned *Rhaphidophora* were committed to a new Saccardian genus *Ophioceras*, 63 species were compiled in *Ophiobolus*, and additions in subsequent volumes have more than trebled this number, even if allowance is made for the 7 species with more or less chaetose perithecia transferred to another Saccardian genus, *Ophiochaeta*, in 1895.

The very considerable increase in number of species assigned to it could hardly have failed to bring into relief the rather indiscriminating inclusiveness of the rehabilitated genus. Yet it was more from motives of taxonomic correctness than of expediency that Höhnelt (34), in 1918, submitted a remedy in proposing to reestablish *Entodesmium* and *Leptospora* as genera independent of *Ophiobolus*, assignment of the various species to be governed by their similarity to the 3 historical types, *E. rude*, *L. porphyrogona* and *O. acuminatus*, respectively. From his discussion it may be inferred that he regarded the complete disarticulation of the ascospores prevalent in the first of these types as an essential feature of *Entodesmium*, and median disarticulation between 2 swollen segments set forth in Riess' account of the second type, and also illustrated by Berlese in all the species figured by him under his *Leptosphaeriopsis*, as an essential feature of *Ophiobolus*. Each of these modes of disarticulation would seem to occur in only a relatively small proportion of the species compiled in *Ophiobolus* in the *Sylloge fungorum*, and it seems quite possible that their occurrence here may indeed constitute a feature representative of such thoroughgoing parallelism as distinguishes the members of natural genera. *Leptospora*, however, was apparently left by Höhnelt in a more ambiguous state, for although it was to be constructed about *L. porphyrogona*, it was evidently to serve at the same time as repository for all forms included by Saccardo in *Ophiobolus*

that have spores, whether nodose or not nodose, that on maturation remain filamentous, that is, do not in any normal way become disarticulated. Manifestly this disposition would have transferred to *Leptospora*, with only relatively small reduction, the main mass of forms compiled in *Ophiobolus*. What Rabenhorst had emphasized as a distinctive feature of *Leptospora*, an imputed unicellular condition of the ascospores, was omitted from discussion, and not without justification from factual considerations, since Winter had described the spores of *O. porphyrogonus* in at least their later stages as being provided with many cross walls.

In a second paper Höhnelt (35) applied to the genera under discussion the moot structural distinction between discrete fruiting bodies, of which he was a most persistent exponent. Finding the type species of *Leptospora* to be of "sphaeriaceous" structure, he made such structure a requirement for the retention of any fungus in the genus, the forms to be excluded by reason of their "dothideaceous" character being provided for in a new genus, *Leptosporopsis*. Since *Ophiobolus herpotrichus* also was revealed as being "ganz deutlich dothideal," it may be inferred that inclusion in *Leptosporopsis* is to be counted among the events in the taxonomic history of that economically important species. Examination of *Entodesmium rude* likewise showed dothideaceous structure, whereas *O. acuminatus* was recognized as truly sphaeriaceous,—these findings, to be sure, not altering the narrow limits adopted by Höhnelt for the 2 genera concerned in them. A rather considerable widening of the limits of *Ophiobolus* would seem to have been involved, however, in the later recommendation by Weese (78) that the species with 1 or 2 nodes and not given to disarticulation, which Höhnelt had preferred to include in *Leptospora*, be transferred to the former genus.

The dispositions of Höhnelt and of Weese appear to have proceeded from an assumption that if the species in *Ophiobolus* as applied by Saccardo were distributed judiciously mainly among the 3 historical genera and the new *Leptosporopsis* the resulting arrangement would somehow approximate, if not actually constitute, a natural one. Such an assumption would seem at least premature, for, on the basis of our present knowledge, only a relatively small number of the species can with tolerable certainty be assimilated as natural congeners to the type species of the historical genera, leaving, therefore, a large residue of unassimilated forms, which will continue to require an obviously collective genus. Now, since *Ophiobolus* has served as collective genus for a half century, its rehabilitation as a restricted genus would entail shifting of this function elsewhere with numerous changes in established binomials. Considerations of expediency may, therefore, be expected to militate strongly against such rehabilitation, possibly with the result that the group involved may again be integrated, even if



somewhat in defiance of strict priority, in Berlese's genus *Leptosphaeriopsis*. Revival of *Leptospora* and *Entodesmium*, neither of which has in any serious degree been compromised nomenclatorially, would entail no similar difficulty, and might well serve a good purpose, though, evidently, only if through study more detailed than routine examination of mostly dead herbarium specimens, their memberships were maintained in acceptable homogeneity.

Even if the elevation of *Ophiochaeta* to generic rank in 1895 relieved *Ophiobolus* as first applied by Saccardo of only relatively few species, it yet came to affect the nomenclature of both the take-all fungus and the reputed wheat stem killer. Hara (31), in 1916, made reference to the former of these parasites under the binomial *Ophiochaeta graminis* (Sacc.) Hara, and the latter was cited in 1930 by Clements and Shear (4, p. 277) under the binomial *O. herpotricha* (Fr.) Sacc. as the type species of the genus *Ophiochaeta*. The aptness of these dispositions would seem somewhat doubtful, for, judging from Berlese's figures of *Ophiochaeta helminthospora* (Rehm) Sacc. and of *Ophiochaeta penicillus* (Schmidt) Sacc. as well as from the illustrations of *Ophiochaeta chaetophora* (Crouan) Sacc. given by Malbranche and Niel (49), the perithecial setae of at least 3 of the forms Saccardo definitely referred to *Ophiochaeta* were represented by apparently rigid, rather stiffly radiating bristles. Bristles of such character are not readily identified with the flexible filaments that, although often attached here and there to the fruiting bodies of the two graminicolous parasites, have much the appearance of the hyphae that make up the mycelial plate. Berlese (2, v. 2, p. 127) recognized the distinction clearly and held that *O. herpotrichus* could not be referred to *Ophiochaeta*. Nor is it clear that Saccardo ever actually transferred *Ophiobolus herpotrichus* to *Ophiochaeta*, though his statement that this species must be more fully investigated indicates that he had considered such a transfer. In respect to shape of ascospores none of the 3 species of *Ophiochaeta* figured by Berlese and by Malbranche and Niel show close similarity to *Ophiobolus graminis* and *O. herpotrichus*, or for that matter, to one another. The genus as constituted gives the impression of being a heterogeneous assemblage of species and, therefore, as Weese intimated, might perhaps just as well have remained in *Ophiobolus*.

#### MORPHOLOGICAL FEATURES OF THE HELICOID ASCIGEROUS SERIES

In 1925, through the discovery of the ascigerous stage of a fungus causing leaf spot of maize in the warmer regions of the world (13), the genus *Ophiobolus* in the broad Saccardian sense was brought into relation with a second field of phytopathological interest, certainly no less important than the first. The maize parasite directly concerned was one of the rather

numerous series of graminicolous species of *Helminthosporium* distinguished by conidia of typically elongated-elliptical outline that germinate by the production of 2 polar germ tubes, one from the apex, and the other from a narrow zone immediately surrounding the basal scar. As the thoroughgoing parallelism between most known members of the series was fairly obvious from their morphology as well as from their cultural and pathogenic behavior, it was inferred that, when perfect stages should be discovered for other members of the series, they very probably would show intimate morphological similarity with the one then described as *Ophiobolus heterostrophus* Drechsl. The correctness of this inference has since been confirmed. In 1927 Ito and Kuribayashi (38) described under the binomial *Ophiobolus miyabeanus* the ascigerous stage of *H. oryzae* B. de H. A year later Nisikado (57) described under the binomial *Ophiobolus kusanoi* the perfect stage of a fungus causing leaf blight of *Eragrostis major* Host, the conidial condition of which was referred to as *H. kusanoi*. In 1929 Ito and Kuribayashi (42) issued the description of *Ophiobolus sativus*, the ascigerous condition of the widely destructive *H. sativum* Pamm., King and Bakke and, in 1930, followed this with a description (37) of *Ophiobolus setariae*, the ascigerous stage of *H. setariae* Sawada. Martin (51), in a report published in 1931, stated that C. W. Carpenter had obtained the ascigerous stage of the fungus causing brown stripe of sugar cane, *H. stenospilum* Drechsl., and found it to be referable to *Ophiobolus*. As far as can be determined from the rather immature perithecial fructifications that the writer has obtained in cultures of *H. micropus* Drechsl., a parasite on *Paspalum boscianum* Flügge, widely distributed in the Southeastern States, this member of the series provides no departure from the morphological trend of the others.

This morphological trend is manifested conspicuously in pronounced helicoid arrangement of the ascospores within the ascus, taken together with rather unusual dimensional relationships that in part ensue therefrom. Even in *Ophiobolus kusanoi*, the smallest of the 5 ascigerous forms known in detail, the diameter of the ascospore ( $5\mu$ ) exceeds the homologous dimension in the general run of species assigned to *Ophiobolus*, while the ranges of values given for *O. heterostrophus* ( $6-9\mu$ ) are comparable with the asci rather than with the ascospores of a large proportion of the species compiled in the genus, and, indeed, in not a few cases even exceed them. Obviously, an ascus to contain a full complement of 8 such spores would, under any circumstances, need to be of proportionately greater diameter, but here, owing to the additional spatial requirements entailed in the helicoid arrangement of the spores, it needs to be of a diameter exceeding the corresponding dimension of *Ophiobolus* species generally, in a measure, more than proportional to the greater widths of the ascospores. It is not

surprising, therefore, that the published values for diameter of ascus in *O. heterostrophus* (24–28  $\mu$ ), *O. setariae* (22–32  $\mu$ ), *O. miyabeanus* (25–32  $\mu$ ), and *O. sativus* (32–45  $\mu$ ) are unequalled among the corresponding values given under the genus in the *Sylloge Fungorum*, and that only a few of the latter are comparable even with the values (14–18  $\mu$ ) given for *O. kusanoi*. As the spiral arrangement permits the ascospores to attain a length considerably greater than that of the ascus, spores of unusual length (the maximum value given for this dimension in *O. miyabeanus*, 468  $\mu$ , would be regarded as extraordinary in any group of fungi), as well as of respectable diameter are borne in asci that, among scolecosporous groups, appear more remarkable for width than for length.

It is, of course, not to be asserted that the features largely giving character to the ascigerous series under consideration are entirely unknown among the numerous unsifted saprophytic forms compiled in *Ophiobolus*. As was pointed out earlier in the discussion of the parasite causing leaf spot of maize, helicoid arrangement of ascospores, besides being well illustrated in Berlese's figures of *Ophiobolus camptosporus* Sacc., has been attributed to both *Ophiobolus helicosporus* (B. and Br.) Sacc. and to *Ophiobolus galii* Rich., not to mention *Ophiobolus chaetophorus* (Crouan) Sacc., later transferred to *Ophiochaeta*. A fairly pronounced spiral condition with the direction of rotation reversing several times has been observed in especially well-developed material of a fungus collected near Clarendon, Virginia, with exceptionally long asci and longer ascospores, which, otherwise, in a general way, conforms morphologically to the species or series of species customarily referred to as *Ophiobolus porphyrogonus*. However, in none of these forms is the one feature of similarity sustained by others in a degree making for a parallelism indicative of close relationship with the helicoid graminicolous series. The single, median, nodose cells present in the spiral ascospores of *O. camptosporus* precludes a more complete similarity, and the dimensions of this species are hardly of an order to provide a close parallelism. As measurements of *O. chaetophorus*, *O. helicosporus* and *O. galii* have apparently never been published, information in regard to dimensional relationships is not here available. The very slender and conspicuously filamentous spores of *O. porphyrogonus* and of species closely related to it, differ so markedly from those of any of the helicoid graminicolous series that an intimate connection would seem rather definitely out of question. In many species of *Ophiobolus* a spiral tendency is often or occasionally evident in the upper portions of the ascospores, even where these structures are for the most part straight and disposed parallel to one another. Such a tendency represented, for example, in 2 specimens deposited in Mycological Collections of the Bureau of Plant Industry under covers labelled:

Sydow, *Mycotheca germanica*. 2343. *Ophiobolus ulnosporus* (Cke.) Sacc. Auf Stengeln von *Ballota nigra*, Brandenburg: Tamsel. 24. 7. 1927.

Fungi Dakotenses, Brenckle. 237. *Ophiobolus claviger* Harkn. On *Artemisia biennis*. Kulm, N. Dak., Aug. 18, 1913.

rarely entails more than a half turn of rather wide pitch, so that the ascospores are only infrequently if ever appreciably longer than the asci in which they are contained.

#### ASEXUAL REPRODUCTION IN THE HELICOID SERIES AND IN OPHIOBOLUS

Although the series of strongly helicoid graminicolous species thus exhibits fairly distinctive features in the ascigerous stage, it will undoubtedly continue to be known mainly through the conidial stage achieved in the widely familiar group of forms within the genus *Helminthosporium* characterized by the special type of germination to which reference has been made. In general, the conidia here are produced abundantly on the natural substrata, and with few exceptions, also on artificial media, so that membership in the series is ordinarily very easily determined, even though identification of the individual species may require detailed comparisons. Yet the literature on *Ophiobolus*, outside of the publications dealing with *O. heterostrophus*, *O. kusanoi*, *O. miyabeanus*, *O. sativus*, *O. setariae*, and the brown-stripe parasite, makes no mention of any conidial condition suggestive of *Helminthosporium*. Indeed, relatively few contributions on subsidiary stages of any kind are to be found, and of these not all are in as complete harmony as might be desired.

The Tulasne brothers in their account of the fungus or fungi that they treated as *Rhaphidophora herpotricha* included as a stage in the life history set forth therein a pycnidial form producing elongated 6- to 10-celled stylospores measuring 25 to 35  $\mu$  long by 3.5 to 5  $\mu$  wide. It is not apparent that *Hendersonia herpotricha* Sacc. with 8-septate spores, 36  $\mu$  long and 6  $\mu$  wide, later cited by Saccardo as the pycnidial stage of *Ophiobolus herpotrichus*, was intended to have reference to the same sphaeropsidaceous form; or for that matter, whether so intended or not, that it actually had such reference. Hiltner (32), in 1912, asserted his continued belief in the association of a pycnidial form designated by him as *Hendersonia herpotrichoides* Sacc. that had appeared in 1894, following incubation in a damp chamber, on affected wheat plants originating in Saxony that year, with the *Ophiobolus* perithecia that had later developed thereon. Frank (23) considered it likely that a species of *Phoma* that he called *P. tritici* was associated with his *Weizenhalmtöter*. The genetic connection mentioned by Frank has no more received confirmation than has that mentioned by Hiltner; and in both cases uncertainty as to the identity of the species of *Ophio-*

bolus concerned is present as a disturbing consideration. Similarly, dubious circumstances surround the report by Voges (76) of a connection between *O. herpotrichus* and *Acremonium alternatum* Link. On the one hand, as van de Laar strongly hinted, the measurements of length and maximum width of swollen ascospores,  $66-74 \times 4 \mu$ , given by Voges, certainly indicate *O. graminis* rather than *O. herpotrichus*; and on the other, the relationship of *A. alternatum* to either of these species of *Ophiobolus* has never been confirmed. The pure cultures of *O. herpotrichus* isolated by the writer from different lots of quack grass collected in Clarendon, Va., have so far failed to reveal sporulation of any kind, sexual or asexual.

Subsidiary reproductive stages have been attributed also to *Ophiobolus graminis*. McAlpine (46), in 1904, was strongly inclined to regard a pycnidial form newly described by him under the name *Hendersonia graminis*, but later transferred to the genus *Wojnowicia* by Saccardo and D. Saccardo (68, v. 18, p. 367-368), as connected with the take-all fungus. McKinney and Johnson (48), however, on comparing the two forms in pure culture, found no close similarity between them, and were, therefore, led to question the probability of a genetic connection. Guyot's (29) studies later definitely established *Wojnowicia graminis* as a separate fungus. Mangin's claim of a connection between *O. graminis* and a species of *Coniosporium* somewhat doubtfully identified as *C. rhizophylum* (Preuss) Sacc. has likewise remained without confirmation. On the other hand, the production frequently of minute falcate sporidia in the germination of the ascospores of *O. graminis*, first reported by Mangin, was later confirmed by Foex (19), by Kirby (40), and by Foex and Rosella (21).

The Tulasne brothers attributed to the fungus discussed by them under the name *Rhaphidophora carduorum* pycnidia filled with cylindrical curved conidia as well as spermogonia producing cylindrical curved spermatia. Fuckel listed *Phoma acutum* and *Phoma complanatum* under *Rhaphidospora pellita*, *Phoma acutum* under *R. urticae*, and *Phoma dictamni* under *R. dictamni*. Höhnelt (33), in 1915, stated that the asexual stages thus listed by Fuckel certainly did not belong to the ascigerous species to which they had been referred and held as questionable the association of pycnidial and spermogonial fungi with *Ophiobolus herpotrichus* and *O. carduorum* set forth by the Tulasne brothers. He considered as certain a connection between *O. porphyrogonus* and a pycnidial form, *Pleurophoma porphyrogena* Höhnelt, having pycnidia measuring 150 to 250  $\mu$  in diameter that gave rise to rod-shaped hyaline spores 5 to 6.5  $\mu$  in length and 0.8  $\mu$  in width. In the *Sylloge Fungorum*, *Phoma rudis* Sacc. is mentioned, evidently on the authority of Karsten, as possibly a spermogonial phase and *Septoria rudis* Sacc. as possibly a pycnidial stage of *O. rudis*; *Phoma hesperidis* Sacc. is cited as the spermogonial phase of *O. hesperidis* Sacc.;

*Chalara monticellica* Sacc. is indicated as a conidial stage of *O. monticellus* Sacc.; and reference is made to accessory reproductive phases in the diagnoses of *O. glomus* (B. and C.) Sacc., *O. rhagadoli* Passer. and *O. claviger* Harkn.

In the hope that some information might be obtained that would supply a clue as to the merit of these different references to subsidiary stages, the writer collected during May, 1932, living material of about a dozen species of *Ophiobolus* on the dead remains of various coarsely herbaceous plants. Pure cultures of each species were obtained readily by making dilution plate cultures from ascospores crushed out of the perithecia, care being taken to avoid contamination from admixed fungi. All showed satisfactory vegetative development on maize-meal decoction agar. Submerged sclerotia were developed in cultures of several forms of the type usually referred to as *O. porphyrogonus*. Scattered sclerotia representing probably immature fruiting bodies were formed in cultures of the well-characterized species occurring on stems of *Ambrosia trifida* L., the ascospores of which exhibit at their distal end a contour strongly suggestive of the head of a snake. The fungus was identified with the *O. anguillides* (Cooke) Sacc. reported on the same host substratum from Indiana by Fink and Fusan (17) as it was found to agree very well with specimens deposited in Mycological Collections of the Bureau of Plant Industry under the following labels:

Fungi dakotensis, Brenckle. 236. *Ophiobolus anguillides* (Cke.) Sacc.  
On *Ambrosia trifida*. Aug. 24, 1913.

Ascomycetes of Indiana prepared by Bruce Fink and Sylvia Fusan.  
*Ophiobolus anguillides* (Cooke) Sacc. Near Crawfordsville. On  
*Ambrosia trifida*. Number 381. 9-1-1917.

Actual sporulation was observed in cultures of only 2 of the fungi isolated, these having been obtained also from stems of *A. trifida*, on which substratum their fructifications occur year after year in very considerable abundance. Examination of specimens in Mycological Collections showed that the 2 fungi have apparently never been distinguished from one another, each being dealt with separately or both together as *O. fulgidus* (C. and P.) Sacc.

*Sphaeria fulgida* was described from Albany, N. Y., in 1875, evidently on the authority of Clinton and Peck (59) as follows:

"Perithecia gregarious, sometimes disposed in lines, soon free, globose, black, smooth, shining, scarcely papillate, .01'-.012' in diameter, at length collapsed; asci clavate or cylindrical; spores filiform, curved or flexuous, multinucleate, at length multiseptate, colorless, .003' long."

The host substratum was rather indefinitely indicated at the time as being "dead stems of herbs" but not much later, material collected by Peck

at Albany in June, 1879, and distributed under the binomial *Sphaeria fulgida* as No. 583 of Ellis North American Fungi was stated on the label to represent *Artemisia trifida*. It is not known to the writer whether an error was later acknowledged in the identification of the host, but in any case the specimen is now generally filed in herbaria as being of *Ambrosia trifida*, and is evidently thus considered in the host index of Farlow and Seymour (16) as well as in the more recent host index of Seymour (70). There can be little doubt that this disposition is correct, as *Artemisia trifida* Nutt. is not recorded as occurring in the eastern United States and as, moreover, the material is entirely similar to material known to be of *Ambrosia trifida*. A correction in identification of the host genus, impaired somewhat through an apparent garbling of the specific term, would seem to be implied in the citation of *Ambrosia trifolia* as host plant on the label of specimens of *Sphaeria fulgida* collected by Peck in June, 1880, and distributed as No. 1742 of de Thümen's Mycotheca Universalis.

The specimens of *Sphaeria fulgida*, distributed as No. 583 of North American Fungi, contain both of the species of *Ophiobolus* that were found to produce asexual reproductive stages in culture. From the close agreement with respect to size and shape of perithecium (Fig. 1, A, B), to length of ascus (Fig. 1, C), and to length and nearly colorless condition of ascospores (Fig. 1, D, *a-i*) it is evident that the description by Clinton and Peck was based on the smaller of the 2 species. It may be added that the ascospores that are rather regularly and typically 7-septate, and measure 55 to 95  $\mu$  in length by 3 to 3.5  $\mu$  in diameter, when freshly crushed out of the perithecium (Fig. 1, D, *a,b,c,f*) show such swelling in a water mount that a diameter of 4 to 6  $\mu$  (Fig. 1, D, *d,e,g,h,i*) is usually soon attained, the swelling often taking place even when the spores are retained within the ascus. On maize-meal-agar plate cultures the fungus gives rise to a scattering of rather dark, globose pycnidia (Fig. 1, E) from 0.1 mm. to 0.3 mm. in diameter, the exteriors of which reveal pseudoparenchymatous structure. The colorless hyaline pycnospores (Fig. 1, F), which are irregularly ellipsoidal in shape and measure 4 to 7  $\mu$  in length by 2.5 to 4  $\mu$  in width, are liberated from an ostiolar opening situated on the upper side of the pycnidium, sometimes in the center of a crater-like depression.

Although evidently the fungus with 7-septate, nearly colorless ascospores must be regarded as the one to which the binomial *Ophiobolus fulgidus* is correctly to be applied, it is not the one most frequently encountered under this name in herbarium collections. In Mycological Collections of the Bureau of Plant Industry this form has been recognized in only one specimen besides the specimen already referred to, that additional specimen being the one distributed as No. 3824 of the Fungi Columbiana, consisting of old stems of *Ambrosia trifida* collected by J. Dearness at London, On-

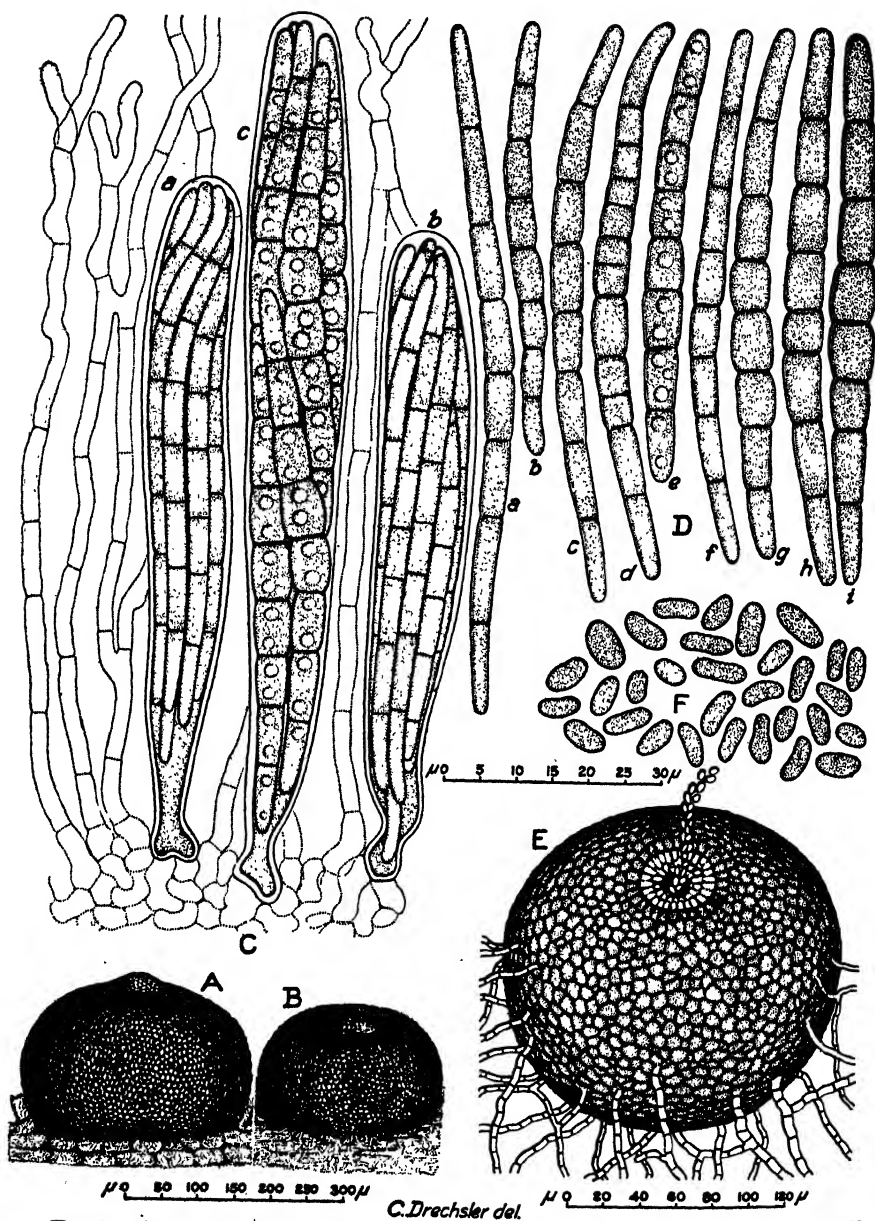


FIG. 1. *Ophiobolus fulgidus*. A. Perithecia on host substratum, viewed in profile.  $\times 100$ . B. Perithecia on host substratum, showing apex somewhat collapsed.  $\times 100$ . C. Asci, the spores within two, *a* and *b*, showing no swelling; the spores within *c* beginning to swell.  $\times 1000$ . D. Ascospores, showing variation in size and shape, *a, b, c*, and *f* not swollen; *d, e, g, h*, and *i* somewhat swollen.  $\times 1000$ . E. Pycnidium produced in pure culture.  $\times 250$ . F. Pycnosporos.  $\times 1000$ .



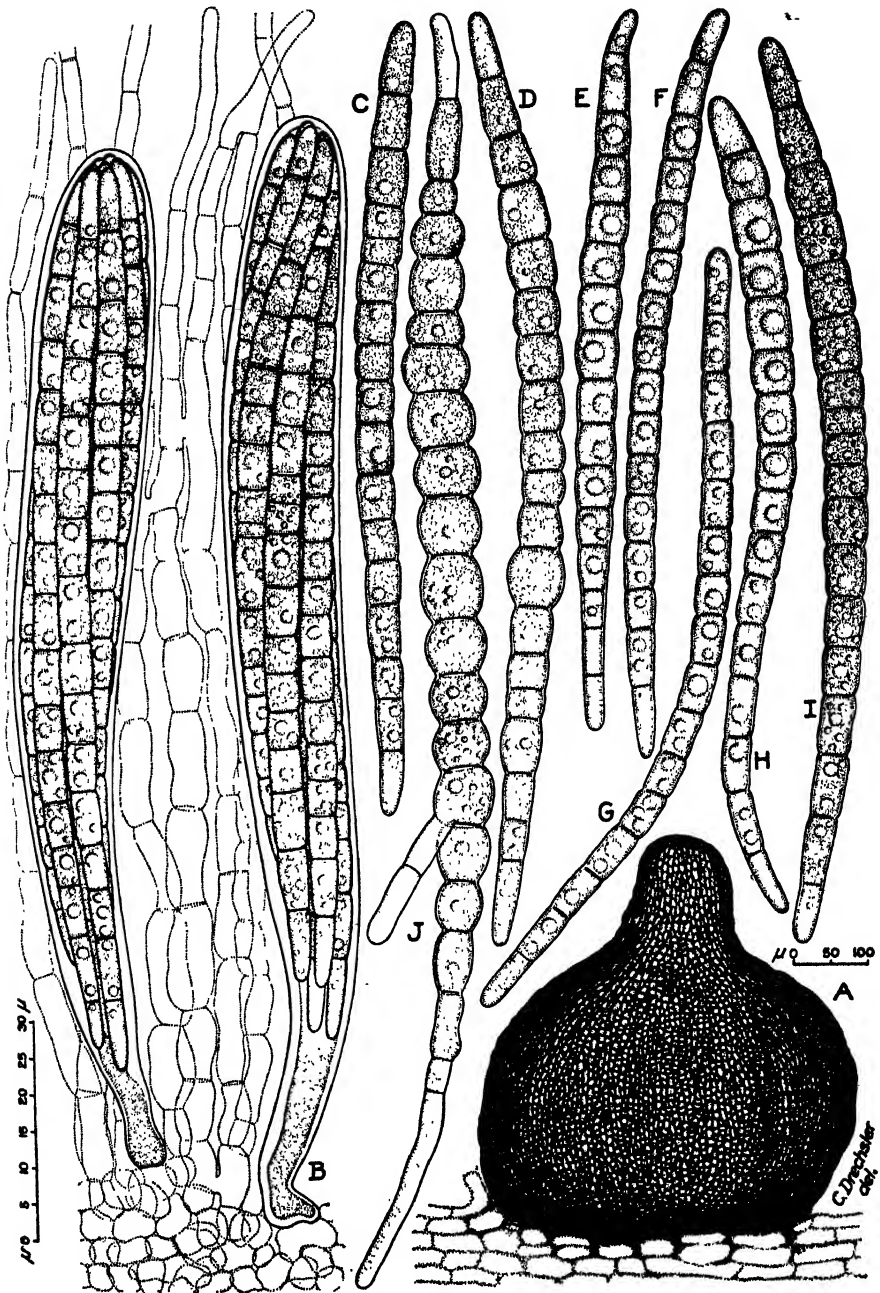


FIG. 2. *Ophiobolus* sp., often identified incorrectly as *O. fulgidus*. A. Perithecium on host substratum in profile.  $\times 100$ . B. Asci, the ascospores not perceptibly swollen.  $\times 1000$ . C-I. Free ascospores.  $\times 1000$ . J. Germinating ascospore.  $\times 1000$ .

tario, May, 1911. Microscope preparations of all other specimens designated as *O. fulgidus* and citing *A. trifida* as host, were found to represent the larger species included in Peck's specimen collected in June, 1879. The globose perithecia here measure mostly .35 to .5 mm. in diameter and are provided usually with a recognizable ostiolar beak, though this modification is generally not so prominent as in the fructification shown in figure 2, A. As there is little tendency toward collapse, the fruiting bodies of this species, which occur often in large numbers in close, somewhat linear arrangement, may be distinguished from those of the first form considered not only by their larger size but also by their more marked protrusion from the substratum. The asci (Fig. 2, B), on maturity, are 100 to 150  $\mu$  long by 13 to 17  $\mu$  wide and contain 8 spores of distinctly yellow coloration, which measure from 85 to 125  $\mu$  in length by 4 to 4.5  $\mu$  in diameter. The spores (Fig. 2, C-I) are usually and typically 15-septate, though the number of cross walls may be as low as 10 or 12 in unusually short individuals, or as high as 18 in unusually long ones. In a water mount living spores, after being liberated from the perithecium, swell to a diameter of 7 or 8  $\mu$ , evidently as a preliminary step toward germination, which ensues generally within a few hours (Fig. 2, J).

In pure culture on maize-meal-agar plates the fungus with 15-septate ascospores exhibits 2 subsidiary reproductive stages. Tawny pycnidia (Fig. 3, A, B) measuring from 25 to 125  $\mu$  in diameter are formed usually more or less superficially on the substratum, mostly in groups readily visible to the naked eye as flesh-colored masses. The pycnidia show externally a pseudoparenchymatous structure, though often and more especially in the smaller individuals (Fig. 3, B) the mycelial origin of this structure may be rather readily apparent. The orifice through which the spores are extruded in a cohesive mass is sometimes surrounded by a fringe of hyphal processes of variable lengths (Fig. 3, A). The pycnospores (Fig. 3, B), which are hyaline, colorless, and ellipsoidal, measure usually 2 to 3.5  $\mu$  in length by 1  $\mu$  in diameter and would seem, therefore, to be somewhat smaller than those of *Phyllosticta ambrosiae*, described by Davis (8) as causing a leaf spot of the giant ragweed in Wisconsin, though the difference in size is hardly sufficient to dismiss entirely the possibility of identity.

A second type of asexual reproduction (Fig. 3, C, D, E) is found in the production of spherical unicellular conidia in usually somewhat tortuous chains terminally on aerial hyphal elements that are mostly not well differentiated from the aerial mycelium generally. Rather curiously, the sporulating elements here are not of approximately uniform widths, as in most similar fungi, but vary in diameter from 1.5 to 4  $\mu$ . With this variation in the conidiophorous hyphae is associated a corresponding variation in the conidia, the more delicate hyphal elements bearing chains of conidia

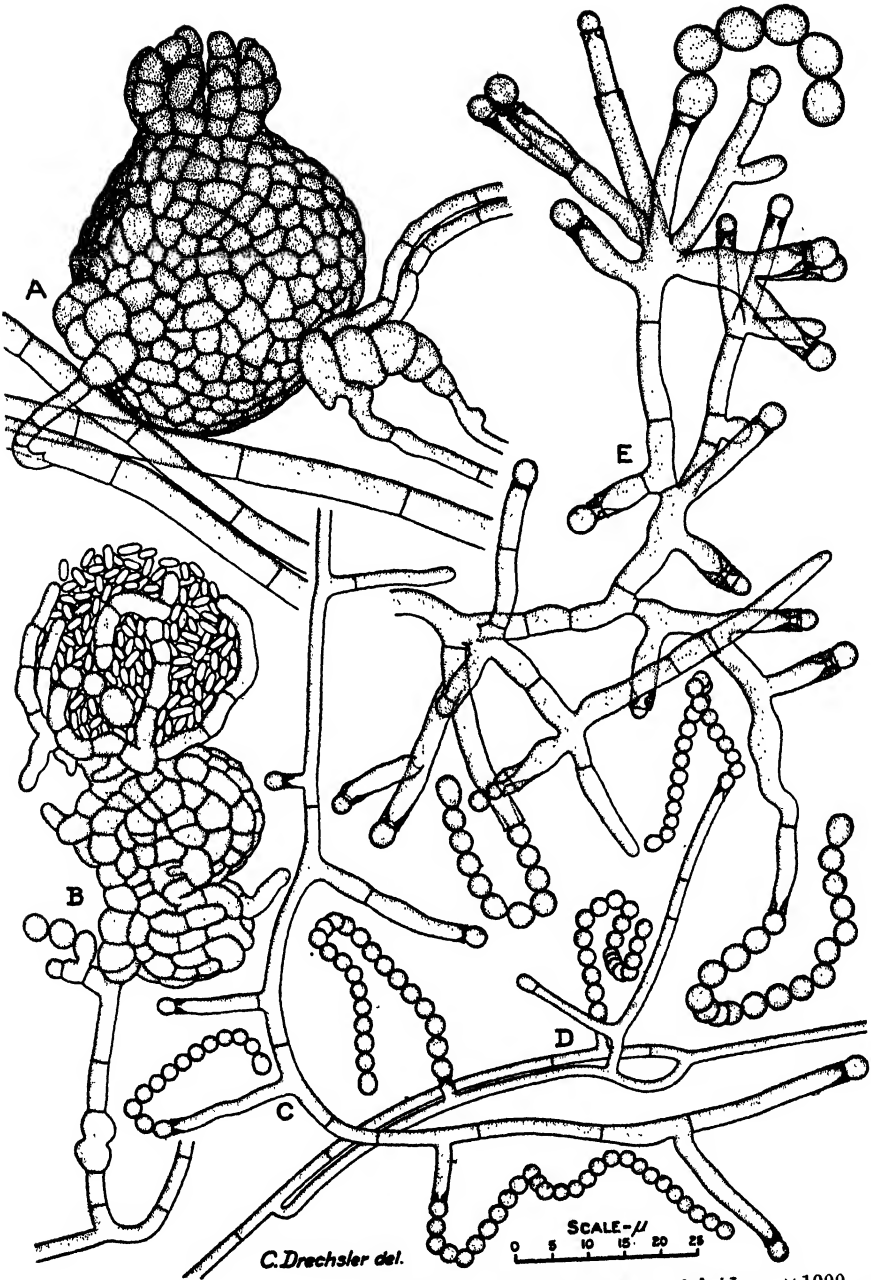


FIG. 3. *Ophiobolus* sp., often identified incorrectly as *O. fulgidus*.  $\times 1000$ . A. Pycnidium of moderate size developed in pure culture. B. Small pycnidium. C-E. Aerial hyphae with sporiferous branches producing chains of aerial sub-spherical conidia, showing cuplike character of some sporulating tips, development of several successive rings at tips, and variation in diameters of conidia and conidiophorous branches; only the basal spore of a chain being shown in most cases.

2  $\mu$  in diameter, whereas the sturdier ones give rise to chains composed of individuals up to 6  $\mu$  in diameter. The production of each successive conidium following the first, entails a characteristic modification of the hyphal tip that becomes increasingly pronounced as the process is repeated again and again. On each occasion the peripheral wall at the tip for a short distance downward is slightly thickened on the inside, with the result that a thick rim soon comes to surround a narrowing isthmus through which the new spores are protruded as buds, one after another. Often the isthmus comes to recede into the cup-like rim, so that the conidia are formed, as it were, partly endogenously, somewhat after the manner described by Andrus and Harter (1) for *Ceratostomella fimbriata* (Ell. and Hals.) Elliott, or by Woronin (80) for *Sordaria coprophila* De Not. Not infrequently, after the lumen of a sporulating tip has become much reduced, the hypha grows out vegetatively a short distance, establishing a new apex in which internal thickening again takes place. As a noticeable ring or collar remains to mark the position of the earlier tip, repetition of the process results in an annulated termination somewhat reminiscent of the conidiophores figured by Ducomet (15, pl. 31) for *Fusicladium dendriticum* (Wallr.) Fuckel, the conidial stage of *Venturia inequalis* (Cooke) Aderhold.

It would, in all probability, be going too far to interpret the tendency toward endogenous development of conidia just described as evidence of parallelism with the association supposed to obtain between *Chalara monticellica* and *Ophiobolus monticellicus*. As chains of conidia are usually much too fragile to withstand the handling incident to collection and removal to a laboratory, circumstances have been generally unfavorable for the recognition of catenulate sporulating stages as associated with ascigerous fructifications on material gathered in field or forest. On the other hand, as has been noted, homologues of the more durable pycnidia found produced in pure cultures of the 2 species cited under the binomial *Ophiobolus fulgidus*, have been recorded in the literature for various species of *Ophiobolus* as members of the form-genus *Phoma*. Genetic relationship with *Phoma* and allied sphaeropsidaceous types may thus represent an attribute shared rather widely among the many fungi occurring on the dead remains of coarsely herbaceous plants that provide the bulk of species subsumed under *Ophiobolus*. Since *Phoma* and the essentially similar *Phyllosticta* include numerous forms parasitic on the higher plants, it is possible that some of the unsifted species of *Ophiobolus*, now known as saprophytes, may ultimately be revealed as disease-producing organisms.

#### THE HELICOID ASCIGEROUS SERIES SET APART AS A NEW GENUS

##### COCHLIOBOLUS

In any case it appears significant that none of the dozen or more species of *Ophiobolus* grown in pure culture from ascospores gave rise to any co-

nidial stage resembling the series of graminicolous *Helminthosporium* forms with bipolar germination. As, conversely, this *Helminthosporium* series has never been found connected with species of *Phoma* or with any other sphaeropsidaceous stage, its separateness from what would seem to be the general run of species in *Ophiobolus*, appears fairly obvious, at least with respect to life-history associations. The series furthermore has apparently no intimate connection with the widely known plant pathogens, *O. graminis* and *O. herpotrichus*, even though its members, rather curiously, occur in part as parasites on the same graminaceous hosts, bringing about somewhat similar, though not identical, pathological changes. In order that confusion may be obviated between the helicoid ascigerous series corresponding to the *Helminthosporium* forms on the one hand and the take-all and stem-killing fungi on the other, it seems expedient to set apart the former as a separate genus for which a term referring to the spiral disposition of the ascospores is proposed.

***Cochliobolus*, gen. nov.**

*Perithecia* sparsa, globulosa, coriaceo-membranacea, ostiolo papillato, atra, glabra aut hyphis sterilis vel hyphis conidiophoris vestita. Asci cylindracei, brevi-stipitati, saepius ad maturitatem plus minusve inflati, typice octospori. Sporidia filiformia, multiseptata, hyalina vel praeprimis ad maturitatem flavo-fumaginea, stipata, vehementer et regulariter spiraliter convoluta. Conidiophora rigidula, simplicia vel ramosa, saepius fusca, pluriseptata, geniculata. Conidia typice elongato-ellipsoidea, recta vel curvata, pluriseptata, hyalino-fumiginea vel fusca saepius olivacea, hyphis duabus germinantia quarum altera ex apice altera hilum juxta emergens.

*Perithecia* scattered, black, submembranaceous to subcoriaceous, smooth or covered more or less with flexuous vegetative filaments or with somewhat more bristling conidiophorous hyphae, globose, usually with evident paraboloid or short cylindrical ostiolar beak. Asci subcylindrical, short-stipitate, often becoming more or less distended especially previous to dehiscence. Ascospores 1 to 8 in number, colorless or especially at maturity somewhat colored, filamentous, provided with many septa, crowded, disposed in strongly helicoid arrangement. Conidiophore simple or somewhat branched, mostly olivaceous, septate, producing first conidium some distance from base and following repeated subterminal elongation successive conidia at intervals later often marked by geniculations. Conidia elongated-ellipsoidal or sometimes somewhat fusoid, straight or curved, nearly colorless to deep olivaceous, provided with plural septa, germinating by the production of 2 polar germ-tubes, one from the apex and the other from a zone immediately surrounding the basal scar.

The type species is *Cochliobolus heterostrophus*, nov. comb. (= *Ophio-*

*bolus heterostrophus* Drechsl., Jour. Agr. Research, v. 31, p. 701-726, 1925; and Phytopathology, v. 17, p. 414, 1927).

The new genus thus defined in both its sexual and asexual stages is intended to include only species that through a thoroughgoing parallelism in morphology and life history can safely be regarded as narrowly and naturally congenèric with the parasite causing leaf spot of maize designated as the type species. As undoubtedly the ascigerous stages of more *Helminthosporium* forms with bipolar germination will become known, in addition to the half dozen already described or reported in the literature, the scope of *Cochliobolus* with respect to number of likely members would seem, even with scrupulous application, to be a sufficiently ample one. Beside the 18 graminicolous species occurring in Japan that were cited by Nisikado (58) under his conidial subgenus eu-*Helminthosporium*, with which in the main the proposed ascigerous genus corresponds, the literature of other countries contains references to an approximately equal number of additional *Helminthosporium* forms parasitic on graminaceous hosts that are evidently referable to the same category. The writer has encountered on hosts other than grasses species that have every appearance of belonging here, the rather frequent occurrence of such forms, especially on dead or fading foliage of sedges, giving grounds for a suspicion that the destructiveness occasioned by the series of fungi in question among the Gramineae may in some measure be duplicated among the Cyperaceae. Conidial forms apparently of the same series have also been recognized on dead materials representing a goodly variety of woody and herbaceous plants, after incubation in a damp chamber, their occurrence, on the whole, indicating a fairly widespread distribution in nature, apparently in saprophytic as well as parasitic relationships.

On the other hand it is hardly to be assumed that the numerous species described from woody and herbaceous substrata that make up the main mass of forms compiled in *Helminthosporium* have representation in the series achieving the perfect stage in *Cochliobolus* in any proportion approximating the representation here of the graminicolous species of *Helminthosporium*. For, judging from the illustrations given by different authors, it appears rather improbable that either of the 2 main series that largely account for the graminicolous species account in comparable measure for the large body of unsifted fungi included under *Helminthosporium* in the *Sylloge Fungorum*. It may not be superfluous to emphasize that not all of even the graminicolous species can be assigned to one or the other of the 2 main series. In mode of germination and production of a Hormodendron-like sporulating stage, *Helminthosporium giganteum* H. and W., as was pointed out earlier (14), certainly departs widely from the series having its ascigerous phase in *Pyrenophora* Fries. The demonstration recently by

Tullis (75) of a connection between *H. sigmoideum* Cav. and *Leptosphaeria salvinii* Catt. has brought to light a third ascomycetous relationship within the group. The short sterigmata on the conidiophores of *H. sigmoideum* figured by Tullis, on which the conidia are borne directly, have no counterpart among the generality of graminicolous species of *Helminthosporium* and may, therefore, have value as a characteristic indicating similar pleomorphic association in other conidial forms. Nor are spherical sclerotia of the type represented in *Sclerotium oryzae* Catt., which is now revealed by Tullis as another phase in the life cycle of the same species of *Leptosphaeria*, known among the members of the 2 main graminicolous series. Even the decidedly different cylindrical sclerotia produced in culture by *H. cyclops* Drechsl. arouse misgivings concerning the affinities of that species, though the discrete conidiophores and the conidia here conform tolerably well in morphology to the homologous structures of known species of *Cochliobolus*. Apparently similar columnar or filamentous sclerotia occur frequently in a series of species, including forms like *H. geniculatum* T. and E. and *H. inequale* Shear, that are distinguished further by smaller, often geniculate conidia, the swollen middle cells of which are usually darker than the end segments. Whether the series, which is often recognized on grounds well set forth by Mason (52), as a separate genus, *Acrothecium* Preuss (?), falls outside the scope of *Cochliobolus*, must remain in doubt until the ascigerous condition of one of its members has been discovered. Of the arrangement of the conidia in a terminal whorl, which is customarily cited as the most distinctive feature of the series or genus, the present writer has seen little evidence, since, in all cultures of the group examined by him, the arrangement was found exactly as in cultures, for example, of *H. sativum*. Indeed, to the extent to which any difference was observed, it was rather the smaller species that produced the more prolonged and more abundantly laden spike-like racemose sporophores.

In characterizing the perithecium in *Cochliobolus* as glabrous or as bearing sterile hyphae or even conidiophores, it is intended to dispose of a feature that has been brought into undue relief through the Saccardian practice of using the presence of setae on the fruiting body, or their absence therefrom, for the separation of genera. The criterion very probably acquired its impetus by virtue of its actual merit in instances where the setae represent special structures pertaining definitely to the reproductive body in question; and it undoubtedly continues to serve well wherever such special structures are involved. However, in application even the occasional presence of adventitious hyphal filaments has sometimes been interpreted as constituting a setose condition. In *C. heterostrophus* the perithecium in some cases is thoroughly glabrous, but in other cases it shows a variable development of undifferentiated hyphae as well as of conidio-

phorous filaments. Such development should, of course, not be interpreted as pertaining to the sexual stage at all, but rather as intrusion of the vegetative and the asexual reproductive phases, brought on by appropriate environmental conditions.

#### HELMINTHOSPORIUM, PYRENOPHORA, AND PLEOSPORA

In this connection it may be appropriate to consider the distinction between the genera *Pyrenophora* and *Pleospora* Rab. In an earlier paper (12) the writer transferred the ascigerous stages of *Helminthosporium teres* Sacc., *H. tritici-repentis* Died. and *H. bromi* Died. from the latter to the former genus, though the transfer entailed the making of several new combinations. The change was made not without the knowledge that as competent an authority as Winter (79) had regarded as inadequate for the separate maintenance of the 2 genera, the distinction based on the presence or absence of setae, emphasized in the works of Saccardo (68) and of Lindau (44). Indeed, it was recognized that Winter's view regarding the inadequacy of this distinction in itself had very considerable justification, for, although sterile bristles not readily to be confused with vegetative hyphae were usually found on the perithecia of the 3 fungi mentioned, their irregular occurrence and occasional suppression argued against their interpretation as structures of primary significance. A circumstance disturbing to Winter's dispositions was apparent in that, if the 2 genera were to be treated as a single genus, the term *Pyrenophora* dating from 1849 might deserve preference on the score of priority over the term *Pleospora*, originating in 1857.

However, when the 2 genera are considered in regard to the life history and the perithecial structure represented in each of their respective types, more substantial reasons for their separation are revealed. The genus *Pleospora* was erected on *P. herbarum* (Pers.) Rab., a species that has become widely known in both saprophytic and parasitic relationships. Since the time when Miyabe (53) definitely determined the parasite on onions (*Allium cepa* L.), which host has apparently as good a claim as any to be considered the type source of the species, to be connected with *Macrosporium parasiticum* Thüm., the same or at least very similar conidial form has been repeatedly shown to be the asexual reproductive stage of the fungus by other workers. In short, an altogether sufficient body of evidence—which the present writer had occasion to corroborate successfully—has accumulated, showing that *P. herbarum* has as its conidial stage a *Macrosporium* species of the "sarcinula" type, for which Mason submitted the name *M. sarcinula* Berk. emend Bolle as the most acceptable one.

When the genus *Pyrenophora* was established, Fries (27) listed in it 3 species under the following names: *P. paradoxa* Fr., *P. inclusa* (Kunz sub



Sclerot.) and *P. phaeocomes* Reb. Concerning the first and second of these species not much information has ever become available, and it is very doubtful whether either of the 2 names could now be definitely referred to any particular fungus. As a result *P. phaeocomes* is left as the obvious type of the genus and is properly listed as type by Clements and Shear. The sclerotia of this fungus were described by Rebentisch (64) in 1804 as *Sphaeria phaeocomes*, the host substratum mentioned being withering leaves of *Holcus mollis* L. The habit sketch supplementing the description, showing imbedded sclerotia distributed over the blade of the grass leaf, like the more recent habit sketch of Berlese, is strongly reminiscent of withered leaves of *Bromus inermis* Leyss. bearing sclerotia of *P. bromi*, such as may be observed in southern Wisconsin during late summer and autumn. Herbarium specimens in the Mycological Collections of the Bureau of Plant Industry, representing collections made in recent times in various European localities and consisting of leaves of *Holcus lanatus* L. and *Holcus mollis* with sclerotia in them scattered much after the manner illustrated in Rebentisch's figures, leave no doubt that the relatively ancient species enjoys an exceptionally consistent application. Though the fungus might well be expected to occur on *H. lanatus* in some of our northern States, the writer has not succeeded in obtaining freshly collected living material of it and, therefore, has been unable to ascertain more precisely the details of its life history. Fuckel, who presumably saw favorable material, stated that its conidial stage was similar to that of *Pyrenophora relicina*, without, however, giving further particulars concerning the morphology of the conidial stage of either species. Yet, since he cited *Pleospora polytricha* Tul. as a synonym of his *Pyrenophora relicina*, his statement is manifestly to be interpreted as implying resemblance to the conidial stage set forth in the account of *Pleospora polytricha* given by the French mycologists. The illustrations of conidia and conidiophores in the plate supplementing this account are perhaps most readily referred to a *Helminthosporium* occurring abundantly every year on overwintered oat straw during May and June in Maryland, Delaware, and Virginia in association with a *Pyrenophora* having ascospores with 5 transverse septa. Though the substratum obviously suggests identity of this *Helminthosporium* with *H. avenae* Eidem, the conidia on the overwintered straw are in general smaller ( $40\text{--}100\ \mu \times 12\text{--}15\ \mu$ ), darker (sometimes olivaceous), and less frequently (usually 2 to 7 times) septate than conidia from the leaf-stripe lesions developing on the foliage of oats, often in some abundance, late in June and early in July. In any case the illustrations of the Tulasne brothers leave no doubt that the conidial stage of their *Pleospora polytricha* belongs to the *Helminthosporium* series with indiscriminate germination from any or all segments, familiar as pathogens concerned in the causation of important

diseases of cereal-crop plants. Fuckel's statement thus testifies to a parallelism in the asexual reproductive stage between Rebentisch's historically important species and the disease-producing series under consideration.

The supplementary parallelism in the sexual stage is expressed especially in the hard sclerotoid texture of the immature perithecium, and in the usually delayed and somewhat protracted development of the asci within. These features were very properly cited by Fuckel in his definition of *Pyrenophora*, which must be reckoned as a very happy one, whether or not the texture and development in question, together with peculiarities in internal perithecial structure associated therewith, can be regarded as requiring inclusion of the genus in the Dothidiaceae, or, for that matter, in the Pseudosphaeriaceae of some more recent writers. The intimate relationship of the species which Fuckel definitely referred to the genus, including besides *P. phaeocomes* and *P. relicina*, the collective *P. trichostoma* (Fr.) Fuckel (from which latter Diedicke (11) later correctly segregated a number of integral species, among them the ascigerous stages of *Helminthosporium teres*, *H. bromi*, and *H. tritici-repentis*), was recognized by Saccardo and Winter in retaining them in a subgenus eu-*Pyrenophora*. When Saccardo amalgamated this distinctive group with the more miscellaneous nonsclerotoid setose forms in his subgenus *Chaetoplea*, the larger genus *Pyrenophora* that resulted manifestly represented a collective genus. The recent elevation of *Chaetoplea* to generic rank by Clements (4, p. 275) remedies this undesirable disposition, in effecting the rehabilitation of *Pyrenophora* as a natural genus in the sense in which it was defined and applied by Fuckel. And to this sense it would seem to be highly desirable that future usage and application should rigorously conform.

#### SUMMARY

Of the 2 graminicolous species, *Ophiobolus herpotrichus* and *O. graminis*, through which the genus *Ophiobolus* became widely known among plant pathologists, the former long retained a reputation as a pathogen in excess of its actual destructiveness. The description of *O. graminis* by Saccardo apparently remained for many years unknown to the earlier observers of cereal-crop diseases, and later, when the description must have been known, Frank, presumably from doubts as to the separateness of the fungus in question from the older *O. herpotrichus*, apparently assigned *Ophiobolus* injury in general to the latter species, and brought the composite concept into prominence through the term *Weizenhalmtöter*. The formidable parasitic character implied in the term adhered to the binomial with which Frank brought it into association, long after the separateness of the 2 species which this author manifestly considered identical, was generally admitted.

*Ophiobolus herpotrichus*, though not hitherto reported in the United States, has been found to occur very generally and in quantity on dead stems of quack grass during April, May, and early June in Wisconsin, New York, Maryland, and Virginia.

*Ophiobolus herpotrichus* and *O. graminis* are probably to be regarded as narrowly related members of a series to which *O. oryzinus* also seems to belong. This series would seem to have no intimate relationship to the generality of forms compiled in *Ophiobolus* nor to the apparently equally independent and phytopathologically important helicoid ascigerous series corresponding to the series of forms in *Helminthosporium* with bipolar germination.

Of the general run of species in *Ophiobolus*, more asexual stages are recorded in *Phoma* than in any other genus. Corroborative evidence of the reality of this pleomorphic association was found in the production of pycnidia in pure cultures of 2 species from dead stems of the giant ragweed, both of which have been treated—the smaller one correctly, the larger one incorrectly, even if more frequently—under the binomial *O. fulgidus*. Of more than a dozen miscellaneous *Ophiobolus* species from various herbaceous host substrata, none gave rise in culture to a *Helminthosporium* stage and none showed any family resemblance in cultural and mycelial habit to species of *Helminthosporium*. It is concluded, therefore, that the helicoid ascigerous series constitutes a separate natural genus, which is accordingly described under the name *Cochliobolus*; the fungus causing leaf spot of maize, *C. heterostrophus*, being designated as type species.

The filamentous outgrowths sometimes present on perithecia of *Cochliobolus* are to be regarded as intrusions of the vegetative and the asexual reproductive stages, rather than as pertaining to the ascigerous stage itself. Excessive emphasis on the presence or absence of setose outgrowths as criterion for distinguishing *Pyrenophora* and *Pleospora* has obscured the much more important difference in life histories present here. Recently rehabilitated as a natural genus through the elevation of *Chaetoplea* to generic rank, *Pyrenophora* again conforms to Fuckel's definition, being properly reserved for the hard sclerotoid perithecial forms having their asexual stages in the *Helminthosporium* series with indiscriminate germination corresponding broadly with the subgenus *Cylindro-Helminthosporium* of Nisikado and the genus *Drechslera* of Ito.

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# INHERITANCE OF ABILITY TO LOCALIZE TOBACCO-MOSAIC VIRUS

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There have been many reports of inheritance of resistance to infection with virus diseases in plants. The following may be cited as examples. Allard (1, 2) noted that the hybrid *Nicotiana tabacum* L.  $\times$  *glutinosa* L.<sup>2</sup> failed to show the mottling which is characteristic of *N. tabacum* when infected with tobacco mosaic, responding instead with necrosis like that of *N. glutinosa*. Smith (12) crossed mosaic-susceptible commercial varieties of spinach with a Manchurian spinach which escaped mosaic in the field, and found that resistance to infection was transmitted to the hybrid seedlings, and could be strengthened by selection among the hybrid strains. Lesley (8, 9) observed a tendency to escape curly-top infection in certain dwarf tomato varieties. He failed to transmit this tendency to standard, i.e., non-dwarf, progenies after hybridization of resistant dwarfs with susceptible commercial varieties of standard habit, but recovered it in dwarf strains derived from hybrids. Bennett (3), in studying alpha-curl virus in the hybrid raspberry variety, Columbian, found recovery from disease symptoms, with disappearance of virus and renewal of susceptibility. It was believed that this hybrid was a seedling from the susceptible variety Cuthbert, pollinated by the immune variety Gregg. Mackie and Esau (10) reported resistance to curly top partially correlated with red color in bean hybrids. Porter (11) found hybrids between mosaic-resistant Chinese Long cucumbers and commercial susceptible strains to be susceptible, but less stunted than the susceptible parents.

The present paper describes a dominant Mendelian genetic factor that causes localization of tobacco-mosaic virus in inoculated leaves of garden pepper, *Capsicum frutescens* L., and a similar factor in each of certain *Nicotiana* L. and *Solanum* L. species. Plants possessing the hereditary factor for localization are susceptible to infection with tobacco-mosaic virus, but resistant to spread of virus in their tissues. It is believed that this is the first description of such a genetic factor affecting a virus disease in its plant hosts. The virus of streak disease of maize is influenced while in its insect host, the leafhopper *Cicadulina mbila* Naude, by a dominant, sex-linked, Mendelian factor, described by Storey (13). This dominant factor in the leafhopper confers ability to act as a vector of the disease. In a recent paper Storey (14) has thrown light on the nature of this hereditary charac-

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<sup>2</sup> *Nicotiana glutinosa* was referred to by Allard as *Nicotiana viscosum*.



ter by demonstrating that the recessive-type, *i.e.*, inactive, insects may be made infective by wounding the wall of the intestine immediately before or after the insects are allowed to feed on diseased plant material, or by introducing virus directly into the insects' blood.

#### CAPSICUM FRUTESCENS

##### Types of Response of Pepper Varieties to Infection with Typical Tobacco-mosaic Virus

The majority of garden pepper varieties respond to infection with typical distorting-type tobacco-mosaic virus (5, p. 847) by mottling. In these varieties, yellowish primary lesions develop about 3 days after inoculation, and are followed by chlorosis of young leaves, with a prolonged period of reduced growth rate for the plant as a whole. Sometimes partial defoliation occurs. In time many mottled and distorted new leaves are formed. The yield of fruit of mottled plants is much less than that of healthy plants. As examples of varieties which give a mottling response, there may be cited the *Capsicum frutescens* varieties California Wonder, Celestial, Chinese Giant, Coral Gem Bouquet, Early Giant, Giant Crimson, Golden Dawn, Golden Queen, Hungarian, Large Bell, Oshkosh, Pimiento, Red Cherry, Red Japan Cluster, Ruby King, Spanish Monstrous, Upright Sweet Salad, and World Beater.

A few pepper varieties, on the other hand, respond by localized necrosis, without systemic mottling. Among the varieties tested, only two have consistently shown sharply-defined necrosis at the site of inoculation. These are *Capsicum frutescens* var. Tabasco, and *C. frutescens* var. *minimum* (previously grown under the name of *C. minimum* Blanco, but differing from common varieties of *C. frutescens* no more than these differ among themselves). These peppers are immune from the serious systemic effects of tobacco mosaic, although they are highly susceptible to infection. Many small necrotic lesions develop in inoculated leaves on the 2nd or 3rd day after inoculation. Virus may be recovered from these lesions, but not from green areas between lesions or from uninoculated parts of infected plants. Affected leaves drop off, usually between the 2nd and 5th days. Leaves with many lesions absciss earlier than those with few. The plants are free of virus as soon as all inoculated leaves have fallen. They are susceptible to repeated infection, but eliminate the virus promptly and grow essentially as normal plants.

Peppers giving these two kinds of response will be referred to as mottling-type and necrotic-type plants, respectively. For the purpose of studying the response of hybrids between mottling-type and necrotic-type plants, crosses were made between the mottling-type varieties Ruby King and Golden Dawn, and the two above-described necrotic-type varieties.

### Response of Hybrids to Infection with Typical Tobacco-mosaic Virus

*Capsicum frutescens* var. *Tabasco* × var. *Ruby King*.—Pollen from a plant of the mottling-type variety, Ruby King, was applied to stigmas of emasculated flowers of the necrotic-type variety, Tabasco. Many seeds were secured, but few germinated. Only 2 seedlings survived to blossom; both of these plants showed necrotic primary lesions after inoculation of leaves with typical distorting-type tobacco-mosaic virus. One of the 2 plants set no seed; the other bore many fruits, which were larger than those of the seed parent Tabasco, and when ripe partly pendant, like those of the pollen parent, Ruby King.

From seeds of the one original fertile plant, 48 second-generation seedlings were grown. Of these, 37 showed only necrotic primary lesions after inoculation with tobacco-mosaic virus, but 11 failed to show necrotic primary lesions and subsequently mottled. This reappearance of the mottling response in approximately a fourth of the second-generation hybrid plants suggested the possibility that a simple Mendelian dominant factor might be responsible for ability to localize the virus. The number of observed plants, however, was so small that this approach to a simple Mendelian ratio might have been accidental.

After the symptoms had been observed, the 48 infected plants were all set outdoors in a row in a garden. No attempt was made to prevent cross-pollination by insects. Eleven plants remained mottled and of high tobacco-mosaic virus content until autumn; the remaining 37, having lost their inoculated leaves, proved free of tobacco-mosaic virus. Some of the plants bore many fruits, others few or more. The 11 mottling-type plants bore comparatively few fruits, and, as later results showed, had probably received some pollen from nearby necrotic-type plants. Enough seeds were collected to grow seedlings from 36 necrotic-type plants and from 8 mottling-type plants.

Eleven of the 36 sets of seedlings from necrotic-type plants contained only necrotic-type plants, 1370 in all; the remaining 25 sets of seedlings from necrotic-type plants, a total of 2611, contained both necrotic-type and mottling-type individuals in each set in ratios approximating 3:1 (3.23:1 in the group as a whole). This seemed to indicate that 25 of the 36 necrotic-type  $F_2$  plants had been heterozygous and 11 homozygous for a virus-localizing character. Combining these with the 11 plants that possessed the apparent recessive character, the ratios 11:25:11 were obtained, with one necrotic-type  $F_2$  plant unclassified because of lack of seed. This is in good agreement with the expected 1:2:1 ratios characteristic of inheritance of a unit character in a second hybrid generation.

The 8 sets of seedlings from mottling plants did not contain mottling-type individuals only, as had been expected, but included some necrotic-

type plants. The ratio was 50 necrotic-type to 208 mottling-type plants, or 0.24:1. This ratio is small, and it is believed that the necrotic-type plants occurring in these progenies were the result of natural cross-pollination from necrotic-type plants growing nearby. To eliminate such accidental cross-pollination, 36 mottling-type seedlings from a heterozygous necrotic-type seed-parent were grown in an isolated greenhouse. Seed was obtained from 20 of these, the others setting no fruit. From this seed, 347 seedlings were grown. The seedlings were inoculated with tobacco-mosaic virus; none of them showed primary necrosis, but all developed mottling-type infections.

These results indicate beyond reasonable doubt that in *Capsicum frutescens* the ability to localize the virus of tobacco mosaic is transmitted in inheritance as a simple Mendelian dominant character, the lack of which allows movement of the virus to distant parts of inoculated plants, with resultant blanching, mottling, stunting, and reduction of crop, the symptoms commonly associated with the presence of the mottling type of tobacco-mosaic infection in peppers. The response of heterozygotes was indistinguishable from that of necrotic-type homozygotes. Lesions on inoculated cotyledons of necrotic-type plants resembled those on true leaves. The inoculation of variegated leaves, which occasionally appear on necrotic-type plants, produced approximately as many characteristic necrotic lesions in white, apparently chlorophyll-free, areas as in normal green areas.

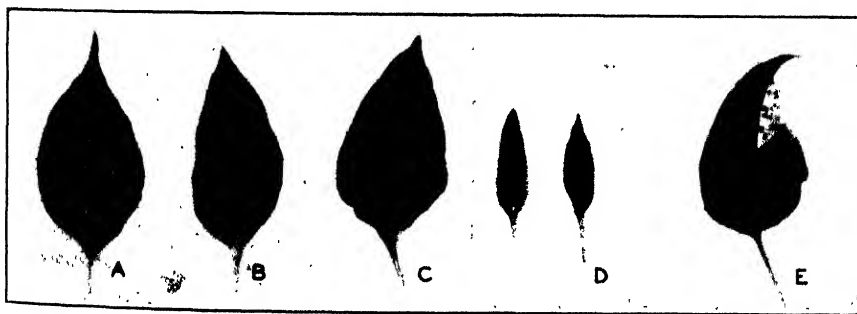


FIG. 1. Leaves of *Capsicum frutescens* inoculated with tobacco-mosaic virus. A. Necrotic primary lesions on leaf of a homozygous plant of constitution, *LL*. B. Lack of such lesions on a similarly treated leaf of a recessive individual, *ll*. C. Necrotic primary lesions on leaf of heterozygous individual, *Ll*. D. Similar necrotic primary lesions on cotyledons of necrotic-type plants. E. Similar lesions in both white and green areas of a variegated leaf.

Figure 1, (A), represents necrotic lesions on an inoculated leaf of a homozygous necrotic-type plant; (B), lack of such lesions on a similarly treated leaf of a mottling-type plant; (C), necrotic lesions on an inoculated

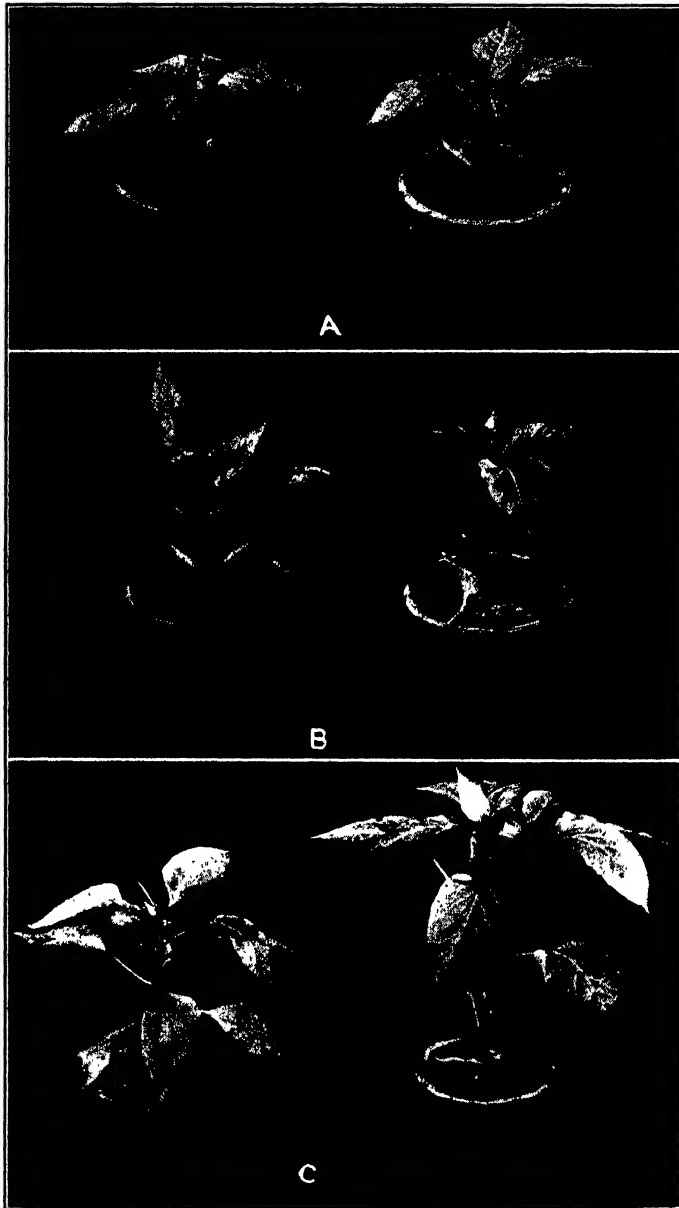


FIG. 2. Two plants of *Capsicum frutescens*, inoculated with tobacco-mosaic virus. The first was a mottling-type plant and the second, a necrotic-type. A. 3 days after inoculation of 2 leaves of each. B. 7 days after inoculation. Inoculated leaves had fallen from necrotic-type plant, freeing it from virus. C. 16 days after inoculation. Mottling-type plant was stunted and mottled. Necrotic-type one was large, without symptoms, and free of virus.

leaf of a heterozygous individual; (D), necrotic primary lesions on cotyledons; and (E), similar primary lesions in both white and green areas of a variegated leaf.

In figure 2 there are represented 2 plants, the first a mottling-type plant, the second a necrotic-type plant, (A) 3 days after inoculation of 2 leaves of each, (B) 7 days after inoculation, when the necrotic-type plant had lost its infected leaves, and (C) 16 days after inoculation, the mottling-type plant being then stunted and mottled, the necrotic-type plant larger, without symptoms of disease, and free of virus.

A fourth hybrid generation was grown, using in most cases seed from commercial, mottling-type *Capsicum frutescens* varieties, such as Ruby King, Golden Dawn, and California Wonder, fertilized with pollen from the third-generation, necrotic-type plants. The seed parents were thus homozygous for the absence of the character for localization, and the pollen parents possessed it either in the homozygous or the heterozygous condition. It was expected, therefore, that each set of seedlings would show either all necrotic-type, or one-half necrotic-type plants. This expectation was realized, for in 8 progenies there were altogether 201 necrotic type and no mottling-type plants, and in 7 progenies in which there were found both types, they were in approximately a 1:1 ratio, 94 seedlings proving to be of the necrotic type and 88 of the mottling type. The fourth generation thus added to the proof previously obtained, that the hereditary factor for localization of virus acts as a simple, dominant Mendelian factor. It also indicated that no incompatibility exists between the factor for localization of tobacco-mosaic virus and certain factors characterizing commercial types of peppers. The plants of this generation resembled commercial varieties in plant and fruit type more nearly than had the first 3 hybrid generations, yet expressed in full the characteristics of the necrotic-type response shown by Tabasco pepper and by plants of the earlier hybrid generations.<sup>3</sup>

*Capsicum frutescens* var. *Golden Dawn* × var. *minimum*.—A study was made of a second hybrid, *Capsicum frutescens* var. *Golden Dawn* × var. *minimum*, in which more known genetic factors were concerned than had been the case with the Tabasco × Ruby King hybrid. *C. frutescens* var. *Golden Dawn* bears white flowers, green immature fruits, pendant, yellow, mild-flavored mature fruits with blunt apices, and is characterized by green cotyledons and hairy stem not branched at base. *C. frutescens* var. *minimum* has purple flowers, blackish-purple immature fruits, erect, red, pungent

<sup>3</sup> Subsequent back-crosses to commercial varieties have yielded individual plants bearing pendant, blunt-nosed, thick-walled, mild-flavored fruits as large as 95 x 50 mm., and reacting to leaf inoculation with tobacco-mosaic virus by formation of necrotic lesions, localization of virus, abscission of inoculated leaves, and subsequent healthy growth, just as in the original Tabasco pepper.

mature fruits with pointed apices, purplish cotyledons, and smooth stem branching from base.

All plants of the first generation of *Capsicum frutescens* var. Golden Dawn  $\times$  var. *minimum* showed purple-edged white flowers, immature fruits tinged with purple, partly pendant, red, pungent, mature fruits with pointed apices, green cotyledons, and nearly smooth stem branching at base. Twenty-six first-generation hybrid plants were grown. They were all inoculated with tobacco-mosaic virus, and all responded like the necrotic-type parent, showing necrotic primary lesions, abscission of inoculated leaves, and subsequent healthy growth.

In  $F_2$  plants grown from seed of selfed  $F_1$  plants an approximate 3:1 ratio between necrotic-type and mottling-type individuals was found, as expected; in a set of 1277 the ratio was 2.93:1. A group of 107 plants was examined for evidence of linkage between the factor for virus localization ( $L$ ) or its recessive allelomorph ( $l$ ) and several previously known genes<sup>4</sup> of the pepper: pungent ( $M$ ) vs. mild ( $m$ ) fruit, pendant ( $P$ ) vs. erect ( $p$ ) fruit, red ( $R$ ) vs. yellow ( $r$ ) mature fruit, purple ( $A$ ) vs. white ( $a$ ) flower, smooth ( $H$ ) vs. hairy ( $h$ ) stems, and pointed ( $D$ ) vs. blunt-nosed ( $d$ ) fruits. No evidence was found suggesting linkage between the gene for localization of tobacco-mosaic virus and any of the others. This is not surprising in view of the fact that there are 12 chromosomes in *Capsicum frutescens*.

Among 109  $F_2$  plants obtained as a result of the back-cross *Capsicum frutescens* var. Golden Dawn  $\times$  (var. Golden Dawn  $\times$  var. *minimum*), 54 proved to be of necrotic type and 55 of mottling type. No evidence was found for linkage between the gene for virus localization and any other.

Similar observations on a set of 113 plants derived by selfing a plant from the earlier Tabasco hybrid stocks gave no evidence of linkage between the factor for green ( $G$ ) vs. pale ( $g$ ) immature fruit and that for virus localization.

Data showing the distribution of the localizing gene and 7 other genes are summarized in table 1.

#### Response to Infection with Other Strains of Tobacco-mosaic Virus

It was found possible to test the response of individual pepper plants to tobacco-mosaic virus without producing systemic infection even of mottling-type plants. This was done by picking a leaf from each plant to be tested, inserting its petiole into a test-tube full of water, and then inoculating the

<sup>4</sup> The genes for purple flower and pendant, red, and pointed fruit are referred to in accordance with the usage of Deshpande, R. B., Studies in Indian chillies. (3) The inheritance of some characters in *Capsicum annum* L. Indian Jour. Agr. Sci. 3: 219-300. 1933.

TABLE 1.—*Absence of linkage between the virus-localizing factor (L) and certain other genetic factors in Capsicum frutescens*

	Factors involved; L (localizing) and X	Observed ratios	Total number of observed plants
	X equals:	LX : Lx : lX : lx	
F <sub>2</sub> from selfed F <sub>1</sub> ; ratios expected in absence of link- age, 9:3:3:1	R (red)	49 : 22 : 26 : 9	106
	M (pungent)	55 : 15 : 21 : 14	105
	A (purple)	53 : 19 : 28 : 7	107
	P (pendant)	54 : 18 : 23 : 11	106
	H (smooth)	52 : 19 : 27 : 8	106
	D (pointed)	56 : 15 : 30 : 5	106
	G (green)	58 : 28 : 21 : 6	113
Back cross; ratios expected in ab- sence of linkage, 1:1:1:1	R (red)	31 : 23 : 23 : 31	108
	M (pungent)	29 : 24 : 34 : 20	107
	A (purple)	27 : 27 : 33 : 22	109
	H (smooth)	27 : 26 : 33 : 22	108

detached leaf. Leaves from necrotic-type plants responded with necrosis as they would if they were still attached to the plants. Leaves from mottling-type plants showed no necrosis, sometimes appearing unaffected by the inoculation, sometimes showing slight chlorosis in primary lesions, or, if yellowing from age, slight chlorophyll retention, as would be expected if the leaves were still attached to the plants.

This detached-leaf test allowed single plants to be tested for possible necrotic response to more than one strain of virus. Successive sets of leaves from plants of known response to the distorting strain of tobacco-mosaic virus were inoculated with the mottling and masked strains of tobacco-mosaic virus described elsewhere (5), and with virus of aucuba mosaic of tomato, propagated in *Nicotiana tabacum*. The set of 109 plants of *Capsicum frutescens* var. Golden Dawn × (var. Golden Dawn × var. *minimum*) already tested by the detached-leaf method for response to infection with the distorting strain of tobacco-mosaic virus, was used to supply leaves for tests with the other virus strains. Two of the plants of the set were not at the time available for this test, and among the 107 tested plants, leaves of which had been inoculated with the distorting strain of tobacco-mosaic virus, the ratio was 52 necrotic (heterozygous for localizing gene, *i.e.*, *Ll* plants) to 55 nonnecrotic (*ll* plants). The same 52 plants that responded with necrosis on leaves inoculated with the distorting strain of tobacco-mosaic virus also showed necrosis of the same sort on additional leaves inoculated with the two mild strains, the mottling and masked strains of tobacco-mosaic virus.

The remaining 55 plants showed no necrosis after inoculation with these strains. Inoculation of a set of leaves with virus of aucuba mosaic of tomato in juice of Turkish tobacco plants induced typical necrotic primary lesions with pale brown center and dark brown periphery on the 52 necrotic-type plants of the set, but the formation of brown, imperfectly necrotic lesions on the leaves of all the mottling-type plants also.

When inoculated leaves were not detached from plants the response to these virus strains was as follows. Inoculation with the masked strain induced slight stunting and mild mottling in *ll* plants, necrosis and leaf abscission, with complete loss of virus in *LL* plants. Inoculation with the mottling strain induced somewhat similar stunting and mottling in *ll* plants, and the usual necrosis, abscission, and loss of virus in *LL* plants. Inoculation with the virus of aucuba mosaic of tomato induced severe stunting, blanching of young leaves, and occasional systemic necrosis in some *ll* plants, but imperfectly necrotic primary lesions, abscission of inoculated leaves, and loss of virus in others; all *LL* plants showed typical primary necrosis, leaf abscission, and loss of virus.

The similarity of necrotic primary lesions in all necrotic-type plants when infected with these 4 strains of tobacco-mosaic virus suggests that the factor that determines virus localization and necrotic response to infection with the common type of tobacco-mosaic virus may act similarly in response to infections with many, possibly all, strains of this virus.

#### NICOTIANA

The simple Mendelian type of inheritance of the character for virus localization in *Capsicum* L. suggested the possibility that the same or a similar character might occur in other plants that show necrotic lesions when infected with tobacco-mosaic virus. Eight crosses were made between necrotic-type and mottling-type species and varieties of *Nicotiana*. In addition, 2 crosses between pairs of necrotic-type species and 1 cross between a pair of mottling-type species were tested. The number of plants involved was not large, but the response in hybrid progenies was definite, and tended to confirm the results obtained with *Capsicum*. Details of these experiments follow.

*Nicotiana tabacum* (mottling)  $\times$  *glutinosa* (necrotic). One *Nicotiana* cross between a mottling-type and a necrotic-type species was made by Allard (1) in 1914. This was the hybrid *N. tabacum*  $\times$  *glutinosa*, the latter species being known to him not as *N. glutinosa*, but as *N. viscosum*. Allard found that the necrotic response of the pollen parent was transmitted to the first-generation hybrid. In view of the fact that the necrotic response of *N. glutinosa* had not been studied intensively at that time, it seemed desirable to obtain the hybrid again in order to determine whether the first-

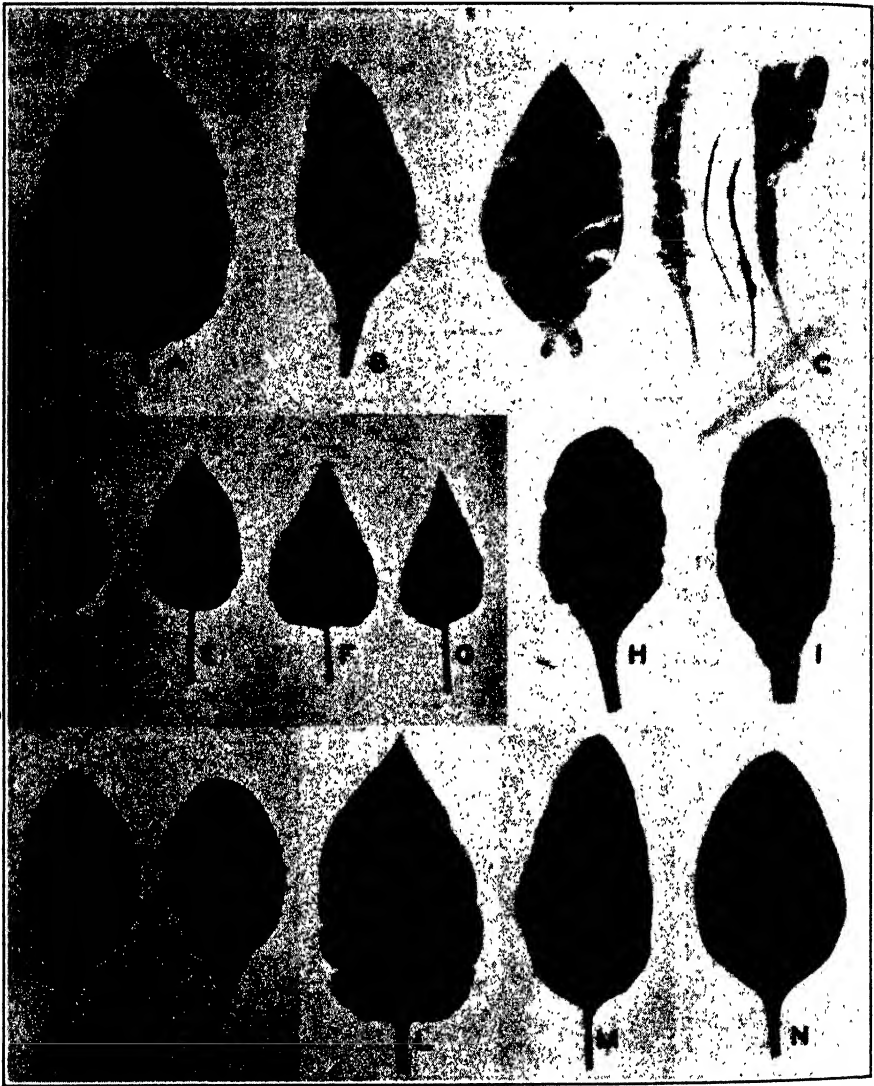


generation plants reproduced the necrotic response of the pollen parent fully, or in modified form.

The variety *purpurea* of the mottling-type species *Nicotiana tabacum* was chosen as seed parent and pollen from *N. glutinosa* was applied to it. The seeds thus obtained germinated well, and the seedlings which grew from them strongly resembled *N. tabacum* in leaf character and flower color. Inoculation of the leaves with tobacco-mosaic virus was followed, however, not by mottling, but by the appearance of necrotic spots closely resembling those produced in *N. glutinosa*. This response was given by all of 319 inoculated  $F_1$  plants. To this extent the necrotic response appeared to be a dominant character, as was the case with the similar necrotic response in the pepper. Unlike the pepper hybrids, however, this *Nicotiana* hybrid was completely sterile, setting no seed during an entire year. For this reason the behavior of the factor responsible for the necrotic response has not been observed in a second or later generation. A photograph of the necrotic primary lesions in the first-generation hybrid is shown in figure 3, A.

*Nicotiana bigelovii* var. *quadrivalvis* (yellowing)  $\times$  *glutinosa* (necrotic). —*N. bigelovii* Wats. var. *quadrivalvis* Pursh. is more severely affected than *N. tabacum* by infection with tobacco-mosaic virus. A prolonged period of stunting of the plant and yellowing of all young leaves precedes formation of mottled and distorted new leaves. The hybrid *N. bigelovii* var. *quadrivalvis*  $\times$  *glutinosa* was tested to determine whether it would respond as had the hybrid *N. tabacum*  $\times$  *glutinosa*. Necrotic primary lesions like those characteristic of *N. glutinosa* appeared on all of 135 inoculated plants of the first-generation hybrid. One hundred similar plants, not inoculated with virus, were grown through an entire summer. Their profuse blossoming did not result in seed formation. Because of this, no evidence was obtained for behavior of the factor for necrosis in later generations. It was evident, however, that in the first generation the necrotic response of *N. glutinosa* was fully dominant over the yellowing and mottling response of *N. bigelovii* var. *quadrivalvis*, as it had been over the mottling response of *N. tabacum* var. *purpurea*.

*Nicotiana bigelovii* var. *quadrivalvis* (yellowing)  $\times$  *sanderæ* (necrotic). —Similar evidence indicating dominance of the factor for necrosis in *N. sanderæ* Sander was obtained from a single plant of *N. bigelovii* var. *quadrivalvis* (yellowing)  $\times$  *sanderæ* (necrotic), which was secured upon attempting to germinate seed formed as a result of the appropriate pollination. The hybrid bore large reddish-purple blossoms, but did not form seeds; its leaves resembled those of *N. bigelovii*, but were of coarser texture. Upon inoculation with tobacco-mosaic virus, it responded by formation of necrotic lesions resembling those of *N. sanderæ* (Fig. 3, B).



• FIG. 3. A and B. Inoculated leaves. A. *Nicotiana tabacum* var. *purpurea* × *glutinosa*. B. *N. bigelovii* var. *quadrivalvis* × *sanderæ*. C. Healthy leaf and 4 systemically infected, mottled, and distorted leaves of *N. bigelovii* var. *quadrivalvis* × *tabacum* var. *purpurea*, showing enations. D-N. Inoculated leaves. D. *N. caudigera*. E. *N. acuminata* × *caudigera*. F. *N. acuminata*. G. *N. caudigera* × *acuminata*. H. *N. sanderæ* (mottling) × *langsдорffii*, recessive individual of F<sub>2</sub>. I. *N. sanderæ* (mottling) × *langsдорffii*, necrotic-type individual of F<sub>2</sub>. J. *N. langsдорffii* × *sanderæ* (necrotic). K. *N. sanderæ* (necrotic) × *langsдорffii*. L. *N. glutinosa* × *langsдорffii*. M. *N. paniculata* × *rustica* var. Winnebago. N. *N. glauca* × *langsдорffii*.

*Nicotiana caudigera* (mottling)  $\times$  *acuminata* (necrotic).—Although some *Nicotiana* hybrids formed no viable seeds, it was found possible to obtain several fertile hybrids, in which the responses of second-generation plants could be studied. One instance was that of the fertile hybrid *N. caudigera* Phil.  $\times$  *acuminata* Hook. *N. caudigera* shows intense yellowing and stunting, followed by the production of distorted, mottled leaves after inoculation with tobacco-mosaic virus. *N. acuminata* shows no mottling, but only brown, necrotic primary lesions as a result of infection. The  $F_1$  hybrid, *N. caudigera*  $\times$  *acuminata*, showed necrosis like that of the pollen parent in all of 135 inoculated plants. The first-generation hybrid set seed abundantly. Seed from a single plant of this first generation was planted and germinated well. The seedlings were not uniform in appearance, some growing rapidly, others slowly. This was not unexpected, since *N. acuminata* had been observed to grow much more rapidly than *N. caudigera*. Altogether, 228 plants of the second hybrid generation were grown and inoculated with tobacco-mosaic virus; 193 responded with necrosis like that of *N. acuminata*, 35 with chlorosis like that of *N. caudigera*. The ratio of necrotic to chlorotic plants was therefore 5.5:1. The reciprocal  $F_1$  hybrid, *N. acuminata*  $\times$  *caudigera*, also proved to be of the necrotic type. Inoculated leaves of the two parent species and of reciprocal hybrids of the first generation are shown in figure 3, D to G.

The facts that the first-generation hybrids all showed a necrotic response, and that the second-generation plants were divided into two groups, giving respectively necrotic and chlorotic responses, seem to indicate that in *Nicotiana*, as in *Capsicum*, a dominant factor determines the necrotic response to infection with tobacco-mosaic virus, the corresponding recessive allowing systemic mottling. The reason for the excess of necrotic-type plants beyond the expected 3:1 ratio in the second generation is not known.

*Nicotiana sanderae* (mottling)  $\times$  *langsдорffii* (necrotic).—In commercial lots of seed of the ornamental, red-flowering *N. sanderae*, which originated many years ago from the cross *N. alata* Link & Otto  $\times$  *forgetiana* Sand., the factor determining a necrotic response is typically present, but not invariably so. It is not unusual to obtain plants of *N. sanderae* that mottle when infected with tobacco-mosaic virus. A mottling-type plant of *N. sanderae* var. Crimson King was used as seed parent in crosses with *N. langsдорffii* Schrank, and *N. sanderae* (necrotic), both crosses producing fertile hybrids.

*N. langsдорffii* is not variable in response, as is *N. sanderae*, but responds invariably with necrosis. Only 13 first-generation hybrids were obtained of *N. sanderae* (mottling)  $\times$  *langsдорffii*. Twelve of these responded to infection with tobacco-mosaic virus with necrosis similar to that shown by *N. langsдорffii*. The remaining plant responded with yellowing primary

lesions, and later mottling, but showed evidence of being an actual hybrid by the form of its blossoms, which resembled those of *N. langsdorffii*, as did those of the other  $F_1$  plants. Seed was obtained from the single mottling  $F_1$  plant and from several of the necrotic-type  $F_1$  plants.

A group of 57 plants from seeds of the one exceptional mottling  $F_1$  plant showed typical localized necrosis in 10, which subsequently grew as healthy plants, mottling in 24, and systemic necrosis resulting in death in 23 individuals. The reason for failure of dominance of the necrotic response in the  $F_1$  plant is not known. Some additional genetic factor may have operated in this exceptional case, preventing the necrotic response in the  $F_1$  plant in spite of the presence of the factor for necrosis. The factor for necrosis was shown to have been present by its reappearance in the  $F_2$  generation.

Seedlings derived from one of the 11 necrotic-type  $F_1$  plants, upon inoculation with tobacco-mosaic virus, showed a necrotic-type response in 134 individuals, and a mottling-type response in the remaining 45, giving the ratio 2.98:1 (Fig. 3, H and I). The factor for necrosis in this instance appeared to be dominant in the usual sense.

*Nicotiana sanderae* (mottling)  $\times$  *sanderae* (necrotic).—The same mottling-type plant of *N. sanderae* var. Crimson King used in the cross with *N. langsdorffii*, was used as seed parent in a cross with a necrotic-type *N. sanderae* plant. The  $F_1$  hybrid, *N. sanderae* (mottling)  $\times$  *sanderae* (necrotic) responded to inoculation with tobacco-mosaic virus by showing necrosis in all of 84 inoculated plants. In the second generation, 249 plants were inoculated; 185 responded with necrosis like that of the original pollen parent and of the  $F_1$  plants, and 64 responded with chlorosis like that of the seed parent (ratio 2.88:1). The necrotic response in this cross appeared fully dominant, and no evidence of interference by other genetic factors was observed.

*N. paniculata* (mottling)  $\times$  *rustica* (necrotic). Two other hybrids were produced between species showing necrosis and those not showing necrosis as a result of infection with tobacco-mosaic virus. They were *N. paniculata* L.  $\times$  *rustica* L. and *N. glauca* R. Grah.  $\times$  *langsdorffii*; these were studied because of responses of the seed parents, one of which produces enations and the other of which is symptomless, or nearly so, when infected.

*N. paniculata* responds to infection with tobacco-mosaic virus by mottling, and by forming enations or tissue outgrowths (6). *N. rustica* responds with systemic necrosis when young, and by localized necrosis when older. Twenty-one plants of the first-generation hybrid, *N. paniculata*  $\times$  *rustica* var. *jamaicensis*, all responded with necrosis closely resembling that of the pollen parent; the necrosis was systemic in young plants, but localized in inoculated leaves of older ones. *N. rustica* var. Winnebago shows necrotic

lesions characterized by the formation of more dark brown pigment at the periphery of the lesion than occurs in the variety *jamaicensis*. Eleven plants of the hybrid *N. paniculata*  $\times$  *rustica* var. Winnebago were inoculated, and 10 responded with lesions surrounded with dark brown pigment of the type of the pollen parent (Fig. 3, M). The 11th plant was atypical in appearance, being round-leaved and dwarfed; it also responded with necrosis, but not with dark brown pigment. The difference in type of necrotic lesion found in the two *N. rustica* varieties thus appeared, with the one exception noted, in the first generation hybrids. No sign of the tendency of *N. paniculata* to form enations appeared in any of the hybrids. Although only 32  $F_1$  plants were grown, they agreed with other crosses in producing all necrotic-type plants from mottling and necrotic-type parents.

*Nicotiana glauca* (nearly symptomless)  $\times$  *langsдорffii* (necrotic). *N. glauca* mottles faintly if infected with tobacco-mosaic virus when young. In older plants the virus multiplies well in inoculated leaves, moves toward the top of the plant erratically, and when present in the younger leaves sometimes causes traces of mottling, and sometimes no symptoms. *N. langsдорffii*, as stated before, shows a necrotic response with production of black-brown pigment at the periphery of primary lesions. A set of plants of the first generation of *N. glauca*  $\times$  *langsдорffii* was grown and 198 plants were inoculated; all responded with necrosis (Fig. 3, N) resembling that characteristic of *N. langsдорffii*.

*Nicotiana langsдорffii* (necrotic)  $\times$  *sanderæ* (necrotic). If the apparently similar genetic factors for necrosis were fundamentally unlike in some of the species already mentioned, combining such dominant factors in a single  $F_1$  plant might result in a modified response. Only two pairs of necrotic-type species were tested. The first of these was *N. langsдорffii*  $\times$  *sanderæ* (necrotic). The response of the first-generation hybrid did not differ from that of the parent species; necrotic lesions surrounded by zones of blackish brown pigment (Fig. 3, J) were produced in all of 162  $F_1$  plants. There appeared to be no additive effect upon combining dominant factors from the two parents in individual hybrid plants. The reciprocal hybrid, *N. sanderæ* (necrotic)  $\times$  *langsдорffii* (Fig. 3, K), was also grown and 92 plants, after being inoculated with tobacco-mosaic virus, all showed a necrotic response like that of the parents and of *N. langsдорffii*  $\times$  *sanderæ*. If the genetic factors in the two parents were similar in nature, but not located in corresponding chromosomes, some plants of a second generation should fail to receive the factor for necrotic response from either parent, since there would be independent segregation. In a second generation grown from seeds of selfed *N. sanderæ*  $\times$  *langsдорffii* plants, all of 255 inoculated plants showed necrotic primary lesions of the type shown in the first hybrid generation. The dominant genetic factors determining the necrotic response

in these two *Nicotiana* species acted as though identical, giving no obvious additive effect when combined, and appearing in all  $F_2$  plants.

*Nicotiana glutinosa* (necrotic)  $\times$  *langsdorffii* (necrotic). The second combination of two necrotic-type species was that of *N. glutinosa* and *N. langsdorffii*. Only a single plant of the hybrid *N. glutinosa*  $\times$  *langsdorffii* grew from seed obtained as a result of appropriate pollination. It was intermediate in appearance between the unlike parent species and proved self-sterile and sterile to *N. langsdorffii* pollen. Upon inoculation with tobacco-mosaic virus it formed necrotic primary lesions (Fig. 3, L) closely resembling those of *N. glutinosa*, but surrounded by a somewhat darker brown peripheral zone, only a little less blackish than that characteristic of *N. langsdorffii*.

No evidence was found in the behavior of the two necrotic-type combinations, *N. sanderae* (necrotic)  $\times$  *langsdorffii* and *N. glutinosa*  $\times$  *langsdorffii*, to indicate that the dominant factors determining necrotic response are different in the species *N. sanderae*, *N. langsdorffii*, and *N. glutinosa*.

*Nicotiana bigelovii* var. *quadrivalvis* (yellowing)  $\times$  *tabacum* (mottling).—Since the hybrids of *N. tabacum* and *N. quadrivalvis* with *N. glutinosa* had resulted in  $F_1$  plants of necrotic type, it was desirable to test the hybrid *N. bigelovii* var. *quadrivalvis*  $\times$  *tabacum*, although it seemed unlikely that there would be production of necrotic-type plants without the use of a necrotic-type parent. The first-generation hybrid *N. bigelovii* var. *quadrivalvis*  $\times$  *tabacum* var. *purpurea* was produced and about 100 plants were grown to blossoming, sets of the plants being inoculated at intervals with tobacco-mosaic virus. The hybrid stock responded to infection by stunting, distortion, and mottling. No necrosis appeared at any time. Outgrowths of tissue (enations) from the lower surface of mottled leaves were numerous in the infected plants (Fig. 3, C), although these had been grown in shade, a condition not suitable for formation of enations on plants of *N. paniculata*, as shown by Jensen (6).

Neither the failure to secure plants of a second generation from some *Nicotiana* hybrids, nor the small numbers of  $F_1$  plants obtained from certain crosses need obscure the result of the study of this genus as a whole. No deviation from the parental responses was found when two necrotic-type species were crossed with each other. A summary of tested plants in eight crosses between mottling-type and necrotic-type plants is shown in table 2. Altogether, 917  $F_1$  plants were inoculated with tobacco-mosaic virus; all but one individual responded with necrosis. The single exception had as one parent the rather variable horticultural form known as *N. sanderae*; some interfering genetic character or combination of characters may have been responsible for the anomalous result. From 3 of the 8 crosses a second generation was secured by selfing plants of the  $F_1$  generation. A total of

TABLE 2.—Summary of tests showing behavior of factor for necrosis in *Nicotiana* hybrids

	First hybrid generation		Second hybrid generation		
	Necrotic type plants	Mottling type plants	Necrotic type plants	Mottling type plants	Ratios
<i>Nicotiana tabacum</i> × <i>glutinosa</i> ....	319	0	...	...	...
<i>Nicotiana bigelovii</i> var. <i>quadrivalvis</i> × <i>glutinosa</i> ....	135	0	..	...	...
<i>Nicotiana bigelovii</i> var. <i>quadrivalvis</i> × <i>sanderæ</i> (necrotic) ....	1	0	...	...	...
<i>Nicotiana caudigera</i> × <i>acuminata</i> ..	135	0	193	35	5.5:1
<i>Nicotiana sanderæ</i> (mottling) × <i>langsдорffii</i> ....	12	1	134	45	3.0:1
<i>Nicotiana sanderæ</i> (mottling) × <i>sanderæ</i> (necrotic) .....	84	0	185	64	2.9:1
<i>Nicotiana paniculata</i> × <i>rustica</i> ....	32	0	...	...	...
<i>Nicotiana glauca</i> × <i>langsдорffii</i> ....	198	0	...	...	..
Totals .....	916	1	512	144	3.6:1

656 F<sub>2</sub> plants were inoculated. A considerable excess of necrotic-type plants occurred in the F<sub>2</sub> generation of *N. caudigera* × *acuminata* (193 necrotic-type; 35 mottling-type); the other F<sub>2</sub> sets gave ratios of 3.0:1 and 2.9:1 respectively. The conclusion seems warranted that in the genus *Nicotiana*, as in *Capsicum*, a necrotic response to infection with tobacco-mosaic virus acts as a typically dominant character in certain crosses between necrotic-type and mottling-type forms.

#### SOLANUM MELONGENA

In the genera *Capsicum* and *Nicotiana*, factors for necrotic response to inoculation with tobacco-mosaic virus behaved similarly, being in both cases dominant over factors for mottling response. In the genus *Solanum*, the species *S. melongena* L. (eggplant) shows both necrotic and mottling types of response among its varieties. It seemed probable that in this genus also the factor for necrosis might be dominant over that for mottling. This was not found to be entirely true, however.

*Solanum melongena* var. Peking Green mottles inconspicuously if infected with tobacco-mosaic virus when young; infected plants tend to become symptomless with age. The variety Hangchow Long is symptomless regardless of age at time of inoculation, but allows virus to increase in its tissues and to spread systemically. The variety Black Beauty is killed by systemic necrosis if infected as a small seedling, but localizes the virus completely and shows necrosis only at the site of inoculation on leaves

of older plants. The mottling-type variety Peking Green and the symptomless variety Hangchow Long were both pollinated from the necrotic-type variety Black Beauty.

*Solanum melongena* var. *Peking Green*  $\times$  var. *Black Beauty*.—The first-generation hybrid *S. melongena* var. *Peking Green*  $\times$  var. *Black Beauty* responded to infection with tobacco-mosaic virus in all of 103 inoculated plants by production of faintly yellowish primary lesions. Around most of these lesions, however, flecks of necrosis later appeared, sometimes developing as thin, light brown, necrotic, circular or wavy lines. A faint systemic mottling later developed, again marked by occasional thin, light brown, necrotic concentric rings and other necrotic markings outlining infected areas. The leaves affected by the systemic spread of the virus were characterized by not being so flat as the corresponding leaves of healthy plants; cup-like depressions and slightly rugose leaf surface seemed to be caused by irregularities in rate of expansion of the lamina. The first-generation hybrids tended toward a symptomless condition in later growth. It was evident that the  $F_1$  plants did not respond like either parent.

A second hybrid generation was grown from seed of several plants of the first-generation hybrid. Among the second-generation plants the solidly necrotic, dark brown primary lesions and similar secondary lesions in young plants characteristic of the necrotic-type parent, Black Beauty eggplant, reappeared in some plants. A considerable part of the remaining individuals showed flecks of ring-necrosis such as had characterized infected  $F_1$  plants. It seemed probable that in this *Solanum* hybrid the necrotic response was not wholly dominant, although a single pair of allelomorphs might possibly account for the responses. The heterozygous plants were irregular in time of formation of traces of necrosis. For this reason attention was directed to observing the ratio of fully necrotic  $F_2$  plants to those showing traces of necrosis or none at all. This ratio, 132:333, amounted to about 1:2.5, and so might be considered to support to some extent the hypothesis that a single Mendelian factor for necrosis existed in *Solanum melongena*, as in *Capsicum frutescens*, and some *Nicotiana* species, although in the case of *S. melongena* dominance appeared imperfect, and the heterozygotes could not be distinguished readily from the non-necrotic segregates.

*Solanum melongena* var. *Hangchow Long*  $\times$  var. *Black Beauty*.—The first-generation hybrid *S. melongena* var. *Hangchow Long*  $\times$  var. *Black Beauty* responded to infection with tobacco-mosaic virus in all of 150 inoculated plants by production of yellowish primary lesions peripherally marked with traces of necrosis and by necrotic flecks on younger leaves. The symptoms were similar to those on the  $F_1$  *Peking Green*  $\times$  *Black Beauty* eggplant. In the second generation, a fully necrotic response, like that of *Black Beauty* eggplant, was noted in 199 of 743 inoculated plants. This gives the ratio of fully-necrotic plants to those not fully necrotic or show-



ing no necrosis as about 1:2.7, a slightly closer approach to a 1:3 ratio than had been obtained with the Peking Green  $\times$  Black Beauty hybrids.

This second *Solanum melongena* hybrid confirms the conclusion reached from observation of the preceding form, that the necrotic response in this species is not fully dominant, but is probably of the nature of a Mendelian character, as in *Nicotiana* and *Capsicum* species.

#### DISCUSSION

The characteristic of localizing tobacco-mosaic virus, because of its easy manipulation as an hereditary unit, may be a useful tool in attempting to gain an understanding of the nature of viruses. A plant that possesses the dominant character is able to restrain tobacco-mosaic virus from producing a systemic infection, although without this factor it would be unable to do so. It has been shown in another paper (5) that several distinct strains of tobacco-mosaic virus differ in degree of freedom to spread in systemic infection of young leaves. In an earlier paper (4, p. 341) it was shown that a similar situation was discernible in iodine-stained material of various hosts of the distorting strain of tobacco-mosaic virus. It has been shown by Kunkel (7) that the virus of aucuba mosaic is in many characteristics similar to that of tobacco mosaic, but its restricted to necrotic primary lesions in *N. sylvestris* Spegaz. & Comes, in which tobacco-mosaic virus produces a systemic mottling. It appears that slight differences in strains of host plants, typified by the difference of a single genetic factor in the pepper, and slight differences in strains of virus which are in most respects similar, may both make differences in freedom of virus spread and even the difference between a localized necrotic infection and one of systemic mottling. Physical or chemical characteristics of the virus, as well as of the host plant, are probably concerned in this relationship.

#### SUMMARY

Localization of tobacco-mosaic virus in *Capsicum frutescens*, the garden pepper, was found to be determined by a dominant Mendelian factor. In plants possessing this factor the virus increased in tissues at the site of inoculation and caused the appearance of numerous necrotic spots of small size, with early abscission of the inoculated leaf and subsequent healthy growth of the plant to maturity. The recessive allelomorph allowed systemic spread of virus, stunting of plant, mottling and distortion of leaves, and reduced yield of fruit.

Somewhat similar genetic factors, determining necrotic response, were found in a number of *Nicotiana* species and in *Solanum melongena*.

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# MULTIPLICATION OF THE VIRUSES OF TOBACCO AND AUCUBA MOSAICS IN GROWING EXCISED TOMATO ROOT TIPS

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Stock supplies of plant-disease viruses are at present maintained in several different ways. One common method is to preserve samples of filtered juice from infected plants in a frozen state in a refrigerator. This method is fairly satisfactory for the maintenance of viruses that retain their activity for reasonably long periods of time under such conditions. It is not satisfactory for all viruses, however, since many soon become inactivated, even when frozen.

Another standard method of maintaining stocks is by inoculation of plants kept in greenhouses or in insect-proof cages. Pieces of tissue from such infected plants can be used at any time as a source of inoculum. The virus, under these conditions, is maintained in a state of active multiplication. Greenhouse or caged plants, however, require much space and special attention. Moreover, with plants grown in greenhouses or cages, the possibility of accidental contamination by insect, human, or other agencies is always present. The purity of strains so maintained can, therefore, not be absolutely relied upon. Attempts have been made to grow host plants under isolated, aseptic conditions free from this danger, but the techniques so far developed are rather cumbersome. No simple reliable method of maintaining stocks of plant-disease viruses under conditions of known purity, free from danger of contamination, and in a state of active multiplication, has been developed to date. Such a method would obviously be of value, especially where many different viruses are being studied in the same laboratory.

The active principle of several plant-virus diseases is known to be present in the roots of plants systemically infected with these diseases and to increase in concentration with progress of time after infection. Since it has been shown that root tips of the tomato, a plant that is susceptible to many different virus diseases, are capable of growing apparently indefinitely in an isolated state under controlled environmental conditions (32), it has seemed possible that such roots might prove to be excellent experimental material in which to maintain certain viruses under controlled conditions. Such cultivation of the viruses would depend (1) on the infected roots' growing sufficiently well to maintain cultures, and (2) on the multiplication of virus in the roots. No marked symptoms have

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been observed in roots of diseased plants in spite of the presence and increase of virus in such roots. For this reason it has sometimes been supposed that multiplication of virus does not take place in roots, but that its presence and increase in roots may be due to accumulation of virus produced elsewhere in the plants. The work presented here was planned to test some of these possibilities.

#### EXPERIMENTAL

The stem of a rapidly growing plant of Bonny Best tomato carrying a pronounced systemic infection with tobacco-mosaic disease was cut up into segments. These were thoroughly washed and so suspended, by single threads in 3-liter Erlenmeyer flasks containing a little water, that the cuttings did not touch either the flask or the water. The flasks were plugged with cotton and left on a laboratory table. Roots developed on the basal portions of the cuttings. After 11 days, 18 root tips were carefully removed and placed in 125-ml. Erlenmeyer flasks, prepared as previously described (31). Each flask contained 50 ml. of nutrient. The nutrient used was prepared as previously described (32) and contained the following substances in the concentrations indicated:

Ca(NO <sub>3</sub> ) <sub>2</sub>	0.60 millimols
MgSO <sub>4</sub>	0.30 "
KNO <sub>3</sub>	0.80 "
KCl	0.87 "
KH <sub>2</sub> PO <sub>4</sub>	0.09 "
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	0.006 "
Sucrose	2% (by weight)
Yeast (extract)	0.01%

Of the 18 roots cultured, 7 became infected with bacteria and had to be discarded, 1 failed to grow, 2 made poor growth and were discarded, 6 were accidentally infected with fungi, and 2 remained in good condition. Of the 6 contaminated roots, 2 succumbed, while 4 were recovered in an aseptic condition by cutting away and discarding the contaminated portions. There were thus 6 roots from which to build up a diseased clone. At the end of a week the 6 surviving cultures were cut into pieces approximately 10 mm. long; the apices were transferred to fresh flasks together with such subapical pieces as were desired for additional cultures, and the discarded basal portions were tested for presence of virus by crushing and rubbing into leaves of *Nicotiana glutinosa* L. All 6 roots contained virus, the individual roots producing from 22 to about 500 lesions. A single root tip was selected for further study. It served as the parent stock from which all subsequent cultures were derived.

The clone was allowed to grow under controlled laboratory conditions and was subcultured (32) at weekly intervals for a period of 30 weeks. At

the 4th passage it contained 50 cultures. This number was maintained through passages 4, 5, 6 and 7, after which enough roots were discarded to reduce the number to 25. The latter number was maintained through all subsequent passages.

At the end of each of the first 4 passages, each culture was tested for presence of virus by inoculation of plants of *Nicotiana glutinosa*. In all passages thereafter, with the exception of the 20th and 30th, only every 5th culture was tested. The virus was present in each root tested in every passage, and although the titer, as shown by the mean number of lesions obtained per culture, varied quite widely from passage to passage, the observed variations were in general apparently related to differences in host-tissue growth rates brought about by external factors. This relationship is shown in figure 1.

At the end of the 20th and the 30th passages, inoculations were made from cultures Nos. 5, 10, 15, 20, and 25 into plants of *Nicotiana tabacum* L.

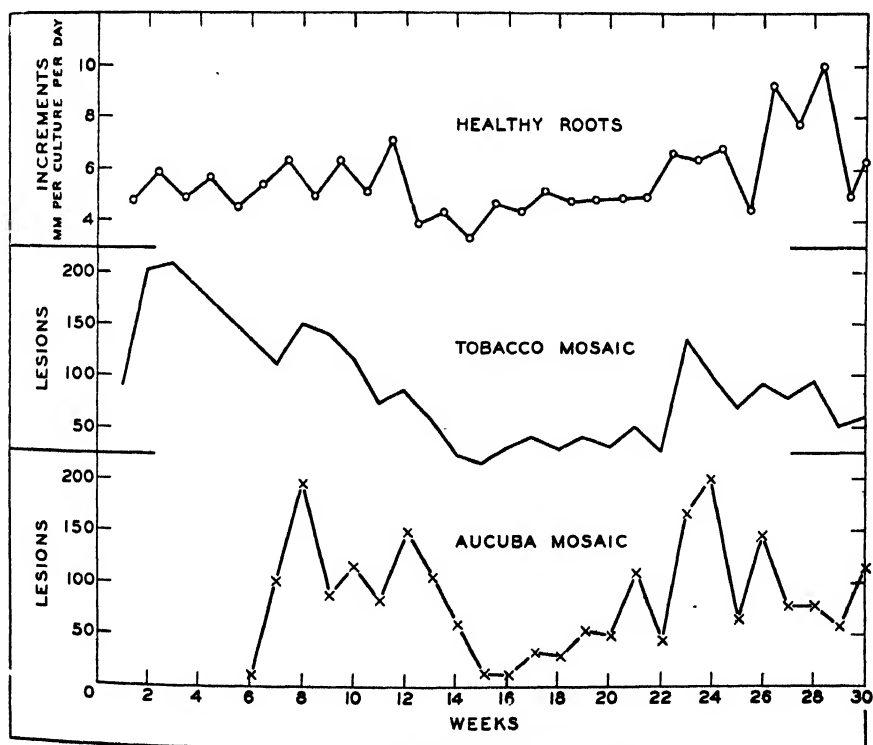


FIG. 1. Weekly variations in titer of tobacco-mosaic virus and aucuba-mosaic virus multiplied in growing isolated root tips and the simultaneous growth rates of healthy roots. The correspondence between the low increment rates from the 12th to the 22nd weeks and the low virus titer obtained in these same weeks is evident.

and *N. sylvestris* Spegaz. and Comes to determine whether the virus was still capable of producing typical symptoms in these plants. At the same time, tissue from each root was inoculated into a leaf of *N. glutinosa* to determine whether virus was present in every culture. At the end of 20 passages, inoculum prepared from every culture except No. 25 induced characteristic local lesions on *N. glutinosa*. Failure in this case was probably due to accidental failure to inoculate the test plant, since the infections produced by other pieces of the same root on plants of *N. tabacum* and *N. sylvestris* were fully systemic and showed no noticeable abnormalities. The results obtained by tests made at the end of 30 passages confirmed those secured by inoculations from roots in the 20th passage. Thirty passages in growing isolated root tips had thus apparently left the virus unchanged in behavior. The theoretical multiplication of host tissue during 30 weeks is estimated to have been of the order  $10^{23}$  (32). Since the virus showed no consistent detectable diminution in titer in this time, it is assumed that its increase had kept pace with the multiplication of the host tissue. The virus is being maintained in further subcultures.

Aucuba mosaic was cultivated in the same way through 25 weekly passages in root tips derived from a single cutting of a systemically infected plant. Virus was recovered from these roots by inoculation into *Nicotiana glutinosa* at the end of every passage. At the end of 20 passages, all roots were tested for the presence of virus by inoculation into *N. glutinosa*, and Nos. 5, 10, 15, 20, and 25 were tested for the characteristic symptoms on *N. tabacum* and *N. sylvestris*. The disease produced in these plants was typical in every instance. The infection on *N. sylvestris* showed the characteristic necrotic local lesions without becoming systemic, while on *N. tabacum* it was systemic and characterized by the usual mottling symptoms. The virus had apparently been unaltered by cultivation in isolated tissues through 20 passages, and, although not subsequently tested for symptom expression, was shown to be present after 25 passages. It is being maintained in further subcultures.

Roots infected with each of these viruses were carefully examined for visible symptoms of disease. They were found to be as straight and clean, with as many and as uniform branches, as were the healthy ones. No macroscopic lesions, discolorations, distortion of tissue, change in manner of growth, or other symptoms of disease, could be observed. It is possible that a detailed histological study may disclose the existence of symptoms not visible macroscopically, but it is a remarkable fact that agents that produce such marked distortion, discoloration, and stunting in the aerial parts of the plant as do these two viruses, should leave the roots, even when carrying large amounts of virus, apparently unaffected. Although growth rates are an unreliable criterion for comparison, unless the different cul-

tures to be compared are all derived from the same clonal parent, it is noteworthy that cultures of the clones carrying these viruses grew as rapidly and appeared in as good condition as did those of healthy clones that were being maintained during the time these experiments were in progress.

Experiments were carried out to determine if virus grown in isolated roots escapes into the surrounding medium or can infect healthy roots when added to the medium and whether healthy roots become infected when wounded in the presence of virus or when grown in the same flasks with and in contact with diseased roots. Four rapidly growing cultures infected with tobacco-mosaic disease were selected at the end of the 3rd passage, and a portion of the nutrient from each flask was tested for presence of virus by rubbing into leaves of *Nicotiana glutinosa*. No lesions were produced by the nutrient from any of the 4, while the roots grown in these flasks produced 39, 40, 28, and 253 lesions, respectively. Ten healthy roots, grown in media to which virus was added, did not become infected. In another group of experiments, 12 rapidly growing roots infected with tobacco-mosaic disease, in the 9th passage, were split lengthwise for about 2 mm. Pieces of healthy roots in the 36th passage (clone C, see 32) were then "threaded" through these diseased pieces. Both diseased and healthy fragments grew actively and at the end of 12 days the pieces were removed and tested separately for presence of virus. The originally healthy fragments in no case produced any lesions, while the originally diseased roots produced from 50 to 200 lesions each (average 114 lesions) on comparable test plants. In still another experiment, roots in the 6th passage carrying tobacco-mosaic virus were placed in the bottom of a Petri dish and crushed with a glass spatula. Ten roots of a healthy clone in the 32nd passage were laid in the juice of these diseased tissues and repeatedly wounded with fine insect pins. They were then removed to fresh nutrient and allowed to grow for one week and subcultured and the discarded portions tested for virus. Five of the 10 roots survived and, when tested, produced a few (1 to 47) lesions on leaves of *Nicotiana glutinosa*. At the end of the 2nd passage after inoculation, however, all of the 16 cultures obtained from the 5 surviving roots were virus-free. In a second series of cultures, 10 roots inoculated in the same way were likewise all virus-free in the second passage. Diseased clones of roots have not been established by direct inoculation. They have only been obtained by growing roots from systemically infected plants. Although the experiments performed are insufficient to warrant the conclusion that roots cannot be infected in these ways, it at least appears that the virus does not ordinarily escape from the diseased tissues into the medium, nor is the disease easily transferred to healthy roots by contact with diseased roots or with media to which virus

has been added. Isolated roots are evidently refractory material as far as inoculation by these methods is concerned.

#### DISCUSSION

It has been shown that the viruses of aucuba mosaic and tobacco mosaic will multiply actively in growing isolated root tips for periods of at least 25 and 30 weeks, respectively. It may be presumed that they can be so maintained indefinitely. The cultures can be kept under strict physiological control, are entirely free from danger of accidental contamination with other viruses, and require comparatively little space. As a means of maintaining stock cultures of viruses, the method appears to present many advantages over methods previously in use. It would, of course, be dangerous to conclude that all plant-disease viruses can be so cultivated.

The absence of symptoms of disease in the roots is worthy of note. It seems possible that this may have its explanation in the chlorophyll-free character of the host tissue used. The pathological picture ordinarily associated with these diseases, the mottling, distortion, and stunting of the aerial parts of the plant, may be referred to the effects of the disease on the chlorophyll apparatus or to disturbances in metabolism resulting from injury to that apparatus. The roots used in these cultures were apparently free of chlorophyll. Photosynthesis, if occurring at all, was reduced to a very low level, far below that necessary for the maintenance of growth (30), but the requisite product of photosynthesis was supplied to the roots in adequate amounts in the form of sucrose added to the nutrient. The roots were thus freed from dependence on photosynthesis and hence from any injury that might be due to disturbance of this process. The fact that under the conditions described the roots showed no ill effects, although carrying considerable quantities of actively multiplying virus, would seem to suggest that the pathogenicity of these viruses is largely due to specific effects on the process of photosynthesis rather than to injurious effects on tissues in general. The increase of virus in these roots makes the hypothesis, sometimes advanced, that multiplication of virus is dependent on the chlorophyll apparatus, seem untenable.

The comparative ease of controlling the experimental conditions under which the viruses multiplied in these cultures adds considerably to the importance of the method. All of the factors constituting the effective environment, insofar as they are understood, can be controlled (30, 31). The method seems favorable for use in studies on the effects of temperature and light on the growth of viruses. The mineral nutrition may be varied in a much simpler manner than is possible when entire plants are used. The organic nutrition of the host tissues can be more easily controlled than by any other method at present available, since the normal products of



photosynthesis can be varied at will without at the same time altering the external conditions that usually control their production.

The cultivation of the viruses of tobacco mosaic and aucuba mosaic in growing isolated root tips is of interest from another point of view. During the last decade, a number of viruses of animal diseases, notably those of foot-and-mouth disease (Hecke, 16, 17, 18; Maitland and Maitland, 21), herpes (Andrewes, 1, 3; Haagen, 14; Parker, 22; Parker and Nye, 24; Rivers, Haagen and Muckenfuss, 27), myxoma (Haagen, 15; Benjamin and Rivers, 4), vaccinia (Carrel and Rivers, 9; Li and Rivers, 19; Maitland and Laing, 20; Rivers and Ward, 25; Rivers, Haagen, and Muckenfuss, 26, 27, 28; Parker and Nye, 23), variola (Haagen, 13, 14), "Virus III" (Andrewes, 1, 2), fowl-pox (Findlay, 12), vesicular stomatitis (Carrel *et al.*, 10), Rous sarcoma (Carrel, 5, 6, 7; Carrel and Ebeling, 8), pseudorabies (Traub, 29), the common cold (Dochez *et al.*, 11), and others, have been multiplied in cultures to which living tissues have been added. In most cases, however, the media employed have been unsuitable for the indefinite multiplication of the host tissues. The viruses have, therefore, been transferred at each passage to fresh pieces of surviving tissue. In fact, the usual practice has been to use as inoculum not a fragment of infected tissue, but a small amount of supernatant after it has been freed of cells by centrifuging or by filtration. The nutrient used in cultures of plant viruses, while adequate for indefinite multiplication of the host tissues, is also simpler than that commonly used in cultures of animal viruses, since it contains no serum, broth, embryonic juice, tissue extract, or other complex organic constituents, with the exception of a very small amount of yeast extract.

#### SUMMARY

A method has been developed for the cultivation of the viruses of tobacco mosaic and aucuba mosaic in isolated root tips grown *in vitro*. The viruses have multiplied actively under controlled, aseptic conditions, in the apparent absence of chlorophyll, and without the production of obvious symptoms. The method provides a simple means of maintaining stocks of these two viruses in a state of active multiplication, yet free from danger of contamination with other viruses.

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# THE MODE OF ENTRANCE OF *USTILAGO ZEA* INTO CORN<sup>1</sup>

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## INTRODUCTION

In recent years we have witnessed an awakening to the importance of losses in corn due to common "boil" smut (*Ustilago zea* (Beckm.) Unger). It is conceded that this ever-present disease annually destroys about two per cent of the corn crop in the United States, and this two per cent amounts to approximately 55,000,000 bushels. There appears to be no practicable method of control other than the breeding of resistant varieties. Therefore, since 1920, much time and energy have been devoted to a study of the disease and the development of resistant varieties of corn.

Stakman and his coworkers (4, 8, 9) have shown that *Ustilago zea* is heterothallic, that some monosporidial or gametic lines are extremely variable, and that in all probability races distinctly different in pathogenicity exist in nature. They have presented evidence that a given line of corn may be resistant to certain combinations of monosporidial lines and susceptible to other combinations. Eddins (5) found that 12 multisporidial cultures from different localities in Iowa differed from each other in their pathogenicity on inbred lines of corn.

Plant breeders have isolated selfed lines of corn highly resistant to smut and have combined certain sweet-corn lines to produce fairly resistant, high-yielding, sweet-corn hybrids. They have met difficulties, however, in the synthesis of highly resistant, high-yielding varieties of field corn, and it was these difficulties which made clear the need for a general study of the factors influencing the development of smut and the nature of resistance of corn to smut. As the method of entrance of *Ustilago zea* has been studied but little since the time of Brefeld, it appeared that a study of penetration was a necessary step to clear the way for studies on the nature of resistance.

Julius Kühn was, according to Brefeld (1), the first to inoculate corn with *Ustilago zea* and observe the entrance of the germ tubes. Kühn reported his observations on one infected plant in a single paragraph in connection with a report of more detailed studies on other smuts. The broad

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<sup>2</sup> This work was done under the direction of Dr. E. C. Stakman, Head of the Section of Plant Pathology. The writer gratefully acknowledges his indebtedness to Dr. Stakman and Drs. J. J. Christensen and J. G. Leach for helpful suggestions and criticisms. The writer also takes great pleasure in acknowledging his indebtedness to Dr. T. C. Loh, Head of the Department of Plant Pathology at Lingnan University, Canton, China, formerly a graduate student at Minnesota, for making most of the drawings presented herewith.

scope of Brefeld's studies on *U. zeae* was perhaps partly due to the many distinct differences between corn smut and closely related smuts of small grains and sorghum. The appearance of galls in all rapidly developing portions of the plant, the sharp localization of the mycelium, and the failure of seedling infection were all in sharp contrast to what he had found in other smuts. Brefeld (2, 3) observed that only tender tissues could be penetrated, that infection is purely local, and that only infections in growing tissues result in galls. He figured the direct penetration of the leaf epidermis by conidial germ tubes. In his discussions of the dissemination of inoculum he stressed "air conidia," or "sporidia" as we now loosely term them, apparently considering that chlamydospores serve merely for the overwintering of the fungus and germinate only by the production of promycelia, from which the sporidia or conidia are budded.

#### MATERIALS AND METHODS

Preliminary studies were made on standard varieties of corn inoculated with combinations of monosporidial lines known to produce galls. At first, Brefeld's method of studying free-hand sections stained with eosin or similar dyes was tried, but with little success. Next, attempts were made to strip the epidermis from the leaves, but again with little success. Then the lactic acid-cotton blue method was tried on leaf-spiral material. This method was superior to the first two, but was not entirely satisfactory because not enough light passes through the entire thickness of leaf for the use of the fairly high magnification necessary for studying penetration by the very small smut hyphae. We therefore resorted to the paraffin method of histological study.

Plants 6 to 15 inches tall were inoculated in the greenhouse, most commonly by the hypodermic-syringe method, using single monosporidial lines, combinations of lines known to produce galls, and chlamydospores in water suspension. Standard varieties of field and sweet corn and selfed lines producing different reactions were used. Material from the leaf-spirals of inoculated plants was killed and fixed on the 2nd, 3rd, 4th, 5th, 6th, and, in a few cases, the 8th days after inoculation. Later, nodal buds or portions of tassel and stalk were killed and fixed as soon as the first symptoms of smut appeared. Material also was killed and fixed from plants inoculated by 2 more nearly natural but less effective methods, namely, (1) spraying the entire plant with a sporidial suspension, and (2) dropping the sporidial suspension into the leaf-spiral.

In the spring of 1932 selected portions were killed and fixed from plants developing the first symptoms of smut under natural conditions. On this material it was much more difficult to find inoculum than on plants inoculated in the greenhouse, and on the latter the loss of time and trouble was

great because the water suspensions of inoculum spread too freely between the rolled leaves. During the summer a method of inoculation that made it much easier to find fairly heavily inoculated surfaces suggested itself. The hypodermic syringe was used to force dry chlamydospores into the "hearts" of the plants. The syringe was set with the plunger half drawn and the needle was dipped into a test tube of dry chlamydospores. Then the needle tip was driven to the "heart" of a plant and the plunger was pressed, forcing the spores from the tip of the needle by a puff of air. This method sometimes scattered chlamydospores a distance of one centimeter from the point of puncture in the tender leaf-spiral tissues, but it left the spores sufficiently massed to enable selection of material with abundant inoculum. Within 48 hours after introduction by this method the spores had germinated well.

Various killing and fixing agents were tried, but Gilson's fluid and Chamberlain's special chromic-acetic-osmic mixture were used most commonly. Numerous stains and combinations of stains also were tried, but Stoughton's (10) Thionin and Orange G combination was perhaps the most satisfactory for differentiating the fungus and host tissues.

During August and September of 1932 considerable time was given to the study of naturally and artificially inoculated silks of various varieties and selfed lines, some of which are very susceptible to ear smut. For this study vital staining with Thionin and immediate examination was depended upon because of the difficulties involved when silks are processed by the paraffin method. On the assumption that infection through the silk might be dependent on fertilization or pollen-tube growth, care was taken in the later trials in this series to supply the inoculated silks with fresh pollen, since shoots in the first stages of silking were cut from plants in the field and placed in laboratory moist chambers. Care also was taken to allow slow drying in some cases, after the spores had started to germinate, for it is known that the thickness of the film of moisture is important in penetration by certain fungi.

#### RESULTS

We have failed to find a single case of peneration of silks by *Ustilago zaeae*, although Brefeld (3) reported that infection of the silk tip is easily observed. However, the common observation of single smut kernels deep in otherwise healthy ears, circumstantial evidence though it is, forces us to consider that the silks may be an avenue of infection, despite the fact that many negative tests were made with varied materials and under varied conditions.

Regarding penetration of leaf tissues, the writer's observations are in fair accord with the statements made by Brefeld 45 years ago. The smut

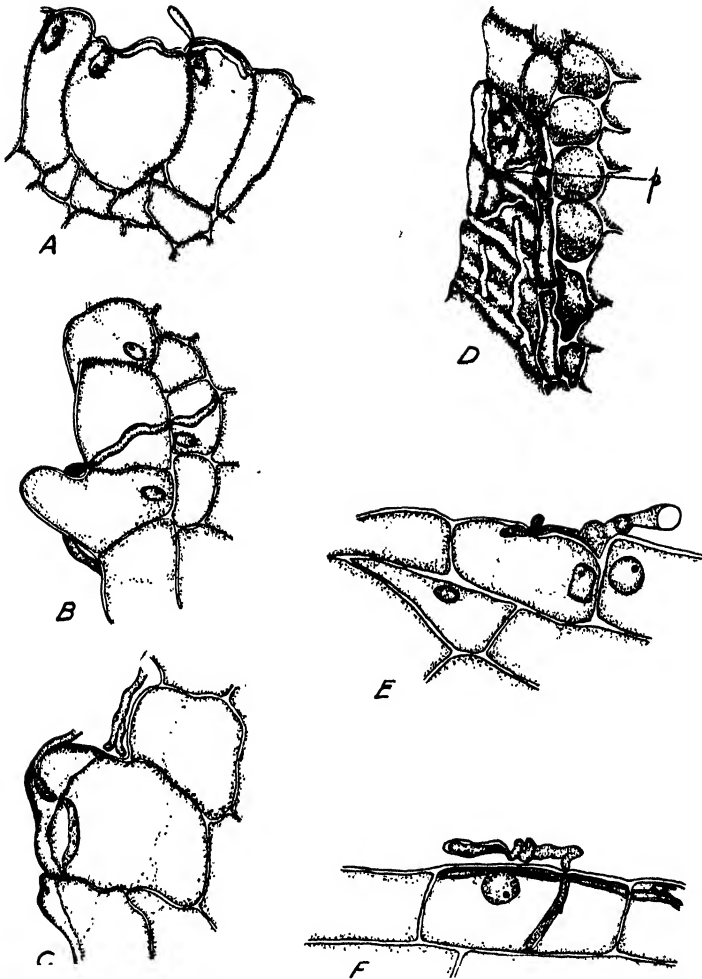


FIG. 1. Freehand drawings of representative penetrations from sporidial germ tubes of *Ustilago zeaе*. Approximately  $\times 800$ . A. Penetration of a cell adjoining a bulliform cell of Rustler (field corn) killed 8 days after inoculation with chlamydo spores in water suspension. Transverse section. B. Penetration near a trichome. Pickaninny (sweet corn) killed 6 days after inoculation with chlamydo spores in water suspension. Transverse section. C. Penetration of a large thin-walled cell by a hypha that is apparently haploid. S-111 killed 12 days after inoculation with a smut-producing combination of monosporidial lines. Transverse section. D. A 3-dimensional view of the necrotic effect of smut hyphae on Golden Bantam corn. Penetration occurred at p. Killed 6 days after inoculation with a smut-producing combination of monosporidial lines. A composite of 3 transverse sections. E. The bulging, curling, and depression-seeking habit of a germ tube, which has recently affected entrance. S-135 (1930) killed 6 days after inoculation with dry chlamydo spores by the syringe method. Longitudinal section. F. The typical curling and swelling of the germ tube. S-135 (1930) killed 6 days after inoculation with dry chlamydo spores by the syringe method. Longitudinal section.

hyphae gain entrance to the plant and pass from cell to cell by direct penetration through the cell walls. In figure 1 there are representative views of penetration by sporidial germ tubes. Many cases of strongly curled, bulged, and knotted hyphae with points pressed against a slightly yielding, perhaps too mature, epidermis have been observed (Figs. 1, B, E, and F, and 2, C). The evidence leads to the conclusion reached by Brefeld that only tender, not yet heavily cuticularized epidermis can be penetrated. The curling and bulging of the hyphae at penetration are strong evidence that the process is mechanical, and the holes through which the infecting hyphae have passed are certainly small (Figs. 1, A, E, and F, and 2, A and C), although it is perhaps to be expected that the openings are constricted considerably by the time the results of the process can be clearly seen. The depressions of the surface of the leaf are the most common points of penetration, and any structures, such as trichomes, which offer the hyphae footing (Fig. 1, B and E), seem to aid them in their attempts to gain entrance.

It must be considered at present that the fungus forces its way into the host in search of nourishment. In their growth along the leaf surface the germ tubes favor the troughs between epidermal cells. In the depressions they bulge, flatten, and press close to the epidermis, but, unlike apressoria, in many cases they constrict again, grow farther, coil sharply, and effect entrance below the coil. The appearance of many penetrations is quite certainly altered by the rapid elongation of host cells sufficiently tender to be penetrated, and entrance is a process that can be observed only in stages and once per subject by the methods used. Figure 1 represents different stages as well as entirely different views of the process.

In many cases the germ tubes of *Ustilago zeae* seem to be attracted by host-cell nuclei, early stages of successful entries and cases of unsuccessful penetration frequently being found with germ tubes contacting the cell wall directly opposite the nucleus. Strangely enough, however, the stomata call forth no response from the germ tubes, and the latter frequently pass directly over the openings to penetrate the nearby cells directly. The hyphae seem to be most attracted by the cells surrounding the guard cells and those in the vicinity of trichomes or bulliform cells. In only 3 cases were hyphae observed penetrating into the substomatal cavity through the stomata; and in all of these there is the possibility that the inoculum had been forced through the openings by the syringe method of inoculation.

Figure 2 is representative of penetration directly from germinating chlamydospores. The latter are perhaps more important as inoculum than Brefeld seemed to think. Examination of material fresh from the field early indicated that the chlamydospores resulting from primary infections are important as secondary inoculum, i.e., for infections on later plants, at least. It is true that smut sporidia usually are abundant in the spiral



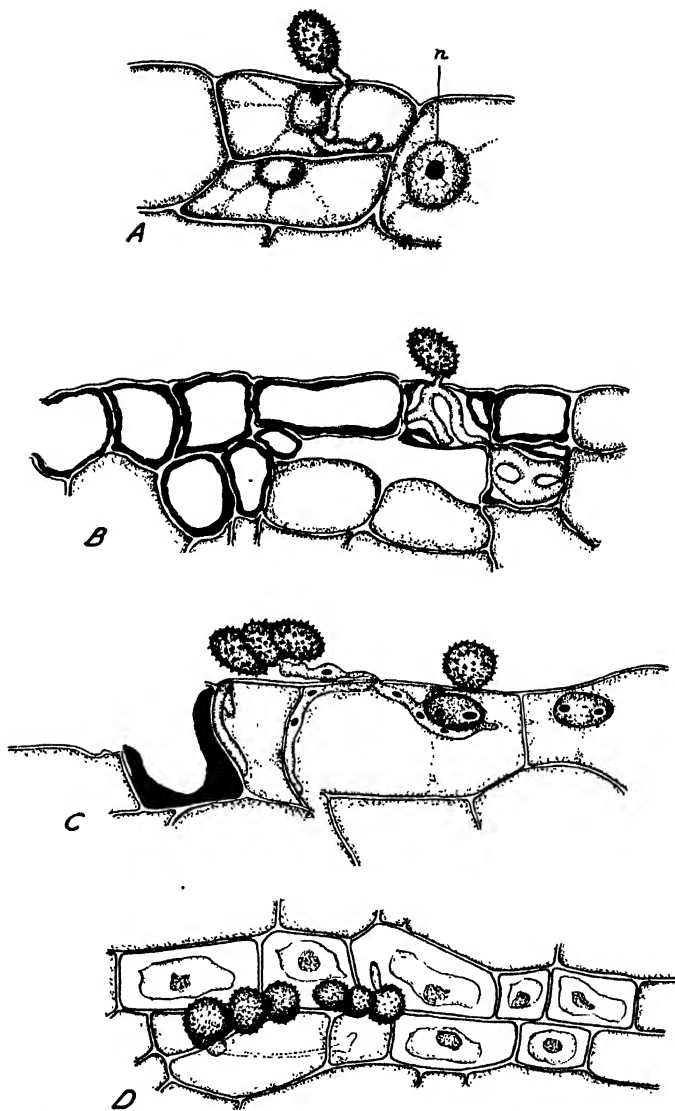


FIG. 2. Freehand drawings of penetrations from chlamydospores. Approximately  $\times 1000$ . A. Penetration of a cell adjoining a guard cell. S-97 (1930) killed 6 days after inoculation with dry chlamydospores. Longitudinal section stained with Heidenhain's iron-alum haematoxylin. Note the enlarged host nucleus, *n*. B. Penetration and necrosis of cells. Rustler killed 8 days after inoculation with chlamydospores in water suspension. Transverse section. C. Penetration and the tendencies of the hyphae to seek contact with host nuclei. This mycelium is representative of that from chlamydospore germ-tube penetrations and it is possibly diploid. S-97 (1930) killed 6 days after inoculation with dry chlamydospores. D. Surface view of penetration and the necrosis of host cells. S-176 (1930) stained fresh with thionin 48 hours after inoculation with dry chlamydospores.

moisture of corn plants if it is easy to find chlamydospores there; but it also is true that it has required less effort to locate penetrations directly from chlamydospores than from sporidia, both having been injected in water suspension. Should it be the case that reduction division does not take place when a germinating chlamydospore sends out an infecting hypha, one would expect, other things being equal, that infections from chlamydospores would be more successful in producing smut galls than infections from haploid sporidia, because the fusion of complementary lines would then not be necessary for the development of the gall. On the other hand, if the aerial conidia that are liberated from the surfaces of infected tissues (Hanna, 6) are diploid or dikaryotic one should expect them to be equally as effective as chlamydospores. Unfortunately, preparations that throw light on these questions have not been secured.

Brefeld (2), Christensen and Stakman (5), and Platz (7) have called attention to the fact that necrosis is an important symptom of smut infection. Since there seemed to be an association between masses of inoculum and necrotic spots in the material studied histologically, a water extract of chlamydospores (soaked 24 hours) and filtrates from broth suspensions of sporidia were injected into the leaf-spirals of a few plants in the greenhouse. There was no evidence whatever of the necrotic effect of smut on these plants and check inoculations produced marked necrosis; hence it seems fair to consider that the necrosis is due to infection or attempted infection by the smut hyphae. The necrosis of host cells not yet reached by infecting hyphae, as well as that of infected cells, is illustrated in figures 1, D, and 2, B and D.

#### DISCUSSION

Although the hyphae of *Ustilago zeae* can penetrate only tender tissue, it is evident, macroscopically as well as microscopically, that they can penetrate and affect tissues which are apparently too mature to produce galls. Meristematic activity and hypertrophy of the host cells are necessary for the formation of the gall or "boil"; and Brefeld's statement (2) that mycelium of *U. zeae* is strictly localized in the host has not yet been qualified or refuted. In view of these facts it seems at present improbable that the mode of entrance of the pathogen can be of great significance in the differential smut reactions of varieties and lines of corn. However, study of a dozen selfed lines and 4 varieties by no means eliminates the possibilities of entrance through stomata, silks, and other deviations from the seemingly general course of mechanical entrance through tender epidermis.

The necrotic effects of smut inoculum require further study, but there are strong indications that necrosis often prevents the development of smut galls. Christensen and Stakman (4) have reported that different collec-

tions of corn smut resulted in different extents of necrosis, depending upon the line of corn inoculated, and it has been commonly observed at the Minnesota station that different combinations of monosporidial lines cause different amounts of necrosis and different amounts of smut on a given line or variety of corn. At present, it seems most reasonable to assume that the necrosis is perhaps a hypersensitive reaction and depends upon the genotypes of both host and attacking fungus. However, the evaluation of this possible type of smut resistance is, as yet, very difficult for the following reasons:

(1) Only 12 selfed lines of different reactions have been studied histologically, and, although there were generalized differences in the extent of necrosis, in certain cases inversely related to the field reaction of the lines, the inoculum was not limited to one combination of monosporidial lines, one genotype of the fungus;

(2) It is assumed that sexual reproduction (reduction division in chlamydospore germination, plasmogamy and karyogamy in the host) frequently and commonly intervenes in the natural propagation of this highly variable fungus, resulting in an infinite number of genotypes in an organism very easily and widely disseminated by the wind;

(3) No line of corn has as yet proved immune from any given smut complement known to produce galls on other lines, and it seems that this would very likely have occurred if resistance were as independent of host-environment interactions and as purely physiological as a hypersensitive reaction would suggest. Worthwhile results on this question can come only through a strictly controlled study of the reactions of different selfed lines, or  $F_1$  generations from homozygous parents, to different smut complements.

#### SUMMARY

*Ustilago zeae* enters the corn plant by direct penetration through the epidermis of young cells.

Chlamydospores, as well as sporidia, may send out germ-tubes that penetrate the host directly.

Attention is called to necrosis as a symptom of smut infection and its possible relation to resistance to *U. zeae*.

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# DISTRIBUTION OF DOWNY MILDEW MYCELIUM IN SPINACH FRUITS<sup>1</sup>

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(Accepted for publication November 20, 1933)

Mycelium of downy mildew, *Peronospora effusa* (Grev.) Tul. (*P. spinaciae* Laub.) was found in abundance in the calyx tube, integument, and nucellus of spinach fruits, *Spinacia oleracea* L. The favorable nature of this material prompted studies to determine the path of invasion of tissues of the ovule and the possible importance of the presence and distribution of mycelium in relation to seed transmission of the disease.

Either mycelium or oospores of downy mildews in the flower or seed parts of certain truck crops has been observed by Clinton (2), Cook (3) (4), Melhus (7) and Leach (6), while the production of infected seedlings from seed suspected of harboring the fungus has been reported by Angell (1) in tobacco and by Leach (6) in beets.

In April and May, 1930, sporulation of *Peronospora effusa* was observed in abundance on the flowers and fruits of spinach. Material then collected has provided an opportunity to study in some detail the tissues of the spinach fruit invaded by the fungus and the paths traversed during the penetration. Recently, Cook (5) reported his observations on the presence of mycelium of spinach mildew in the "pericarp, funiculus and integuments of the ovule." Our studies, in general, confirm his findings and in addition conclusively demonstrate the presence of mycelium in the nucellus.

Some confusion exists in the literature as to the identification of the various structures of the spinach fruit. The outermost part of a spinach fruit is made up of a thickened calyx tube formed by the fusion of two sepals (Stomps (8)). This tube, at maturity, becomes very stony. Inside of the calyx tube and entirely distinct from it is the very thin membranaceous pericarp. At the stage of development shown in figure 1 the pericarp is a thin membrane composed of a few layers of collapsed cells.

The single, basally attached, campylotropous ovule possesses two integuments. In the early stages of ovule development the 2 integuments are clearly differentiated but, at the stage shown in figure 1, they can be recognized as distinct from each other only in the micropylar region. The seed coat formed by the 2 fused integuments encloses the nucellus, a large part of which persists in the mature seed as a storage tissue known as the perisperm.

<sup>1</sup> Contribution from Divisions of Plant Pathology and Botany, Branch of the College of Agriculture, University of California, Davis, California.

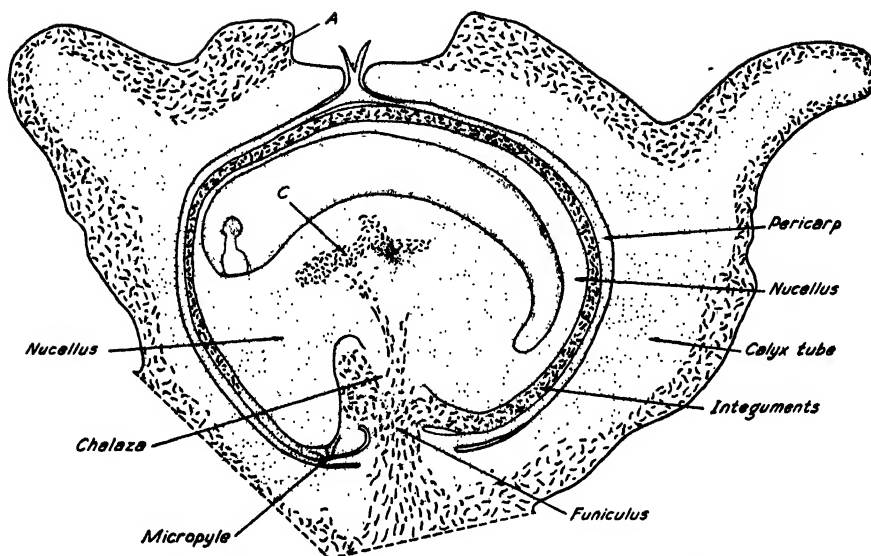


FIG. 1. Diagram of longitudinal section of immature spinach fruit. Distribution of fungus mycelium is shown by irregular broken lines. Letters A and C indicate positions from which sections shown in figure 2, A and C were taken.

The fruits, in which mycelium was found, were collected a few days after fertilization. The embryo at this time had differentiated into a massive multicellular suspensor and globular embryo proper. In the latter there was yet no evidence of the differentiation of cotyledons. Endosperm development at this stage consisted of a single layer of free nuclei lining the embryo sac and enveloping the embryo.

An external examination of the infected plants showed an abundance of conidiophores and conidia on the seed clusters and on localized areas of the leaves, while sporulation or other macroscopic evidence of infection was entirely absent from adjacent areas on the stem. This fact, together with the absence of mycelium from sections of the base of infected clusters, indicates that systemic invasion of seed-bearing branches did not occur. It is probable, therefore, that infection of the fruit occurred through stomata on the calyx tube or pedicel. The mycelium was found in abundance in the outer parts of the calyx tube (Fig. 2, A) while the inner part, which eventually becomes stony, was nearly free of mycelium.

From the base of the calyx tube the mycelium had grown through the funiculus into the integuments (Figs. 1 and 2, D). This, apparently, is the only path of entry to the latter. The inner and outer surfaces of the integuments are covered with definite cutinized membranes that delimit them from the pericarp on the outside and the nucellus on the inside. The fungus mycelium has not been observed to pass through these membranes. Ex-



FIG. 2. A. Mycelium in calyx tube. Section from area A, figure 1.  $\times 144$ . B. Tangential section of ovule showing mycelium in the integuments.  $\times 144$ . C. Mass of mycelium in nucellus. Section from area C, figure 1.  $\times 307$ . D. Longitudinal section through base of ovule, cut in a plane at right angles to that of figure 1. Mycelium is shown passing through the short funiculus into the integuments.  $\times 98$ . *n*, nucellus; *i*, integuments; *f*, funiculus. Arrows indicate location of mycelium.

amination of sections of a number of infected fruits showed that the integuments were uniformly and extensively invaded by mycelium except in the region of the micropyle. A portion of infected seed coat is shown in tangential section in figure 2, B.

The above description represents the extent of invasion found in the majority of infected fruits studied. It is in agreement with the observations of Cook (5), assuming that his so-called "pericarp" is, in reality, the calyx tube. In our studies no mycelium has been found in the membranaceous pericarp, but it seems possible that at certain stages mycelium may gain access.

In one fruit the invasion was not limited to the regions mentioned above but had also involved the nucellus. The mycelium within the funiculus had grown through the chalaza and extended in the form of definitely oriented hyphae upward into the nucellar tissue. Just below the embryo sac these hyphae terminated in a definite area of infected tissue, shown in one plane in figure 1. Examination of the entire series showed that the distribution of the mycelium was limited to a few sections above and below this plane. The hyphae in this area developed into compact masses and appeared to occupy most of the lumen of certain cells (Fig. 2, C). The mycelium was separated from the embryo sac at its closest point by several layers of cells. There was no mycelium in the nucellus in the vicinity of the micropyle or adjacent to the integuments and apparently the only path of entry to the nucellus was through the chalaza.

The distribution of the mycelium in the spinach fruit, as described above, suggested the possibility of seed transmission, and the following studies were made to obtain evidence on this point. Infected flower clusters from the same plants and similar in every way to those used for microscopic studies were tagged and, at maturity, were collected for seed-transmission trials. After being stored through the summer in the laboratory, these seeds were planted in a cool greenhouse in steam-sterilized soil and under isolated conditions. Ten lots of infected seed produced a total of 1260 seedlings, which were kept under observation for 70 days, but no infected seedlings were observed. At the same time 8 lots of seed from infected commercial fields produced 1230 seedlings of which none were infected. Although these trials contributed no positive evidence of seed transmission they were not sufficiently extensive to warrant the conclusion that seed transmission does not occur.

It has been generally observed that seed coats and parts of the fruit wall of onion, beet, and spinach are frequently invaded by the mycelium of certain species of *Peronospora*. No conclusive evidence exists that such mycelium survives within the seed and infects the embryo at the time of germination. The probability of the survival of mycelium would seem to be



enhanced, however, by its presence, as has been here recorded, within the nucellus of the spinach seed.

#### SUMMARY

This paper deals with the presence and distribution of the mycelium of downy mildew (*Peronospora effusa* (Grev.) Tul.) in the fruits of spinach (*Spinacia oleracea* L.). Hyphae were found in the calyx tube, funiculus, integument, and nucellus.

The mycelium enters the ovule through the funiculus and from this point invades the integument. The fungus then spreads throughout most of the integument, thus enveloping the ovule. The nucellus is invaded by mycelium that enters through the chalaza.

No evidence of seed transmission was obtained from limited germination trials with infected seed.

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# A NEW SPECIES OF PESTALOTIA ON PODOCARPUS<sup>1</sup>

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(Accepted for publication October 21, 1933)

In the Autumn of 1932 a bush of *Podocarpus elongata* growing in the Kibble Palace, Glasgow Botanic Gardens, was observed to be suffering from a severe attack of some form of leaf spot. Microscopic examination showed the lesions to be uniformly associated with the presence of a species of *Pestalotia*, the identification of which proved a matter of unexpected difficulty. Reference to Guba's Monograph of the genus *Pestalotia*<sup>3,4</sup> showed it to resemble most closely *P. foedans* Sacc. & Ellis, recorded on the bark of *Chamaecyparis thyoides*. In view, however, of minor divergences in morphological characters and the difference in host genus and organ involved specimens were forwarded to Prof. Guba. After an examination of a pure culture and of diseased leaves, he expressed the opinion that it was an undescribed species.

During the past twelve months the fungus has been kept under observation, and it seems desirable at this stage to put on record such information as has accumulated regarding it.

The leaves of *Pestalotia elongata* may be over 5 inches long but do not normally exceed .25 inch in width. Lesions may develop at any point but are more frequent at or near the apex, where drops of water remain longest unevaporated. When they arise at the tip both sides of the prominent midrib are usually involved, but in the case of lesions originating at other points infection is restricted normally to one side of the midrib. The necrotic areas assume at first a purplish brown tint, which is always retained on the lower side of the leaf. Their upper surface tends to become greyish and even silvery, standing out conspicuously against the dark green of the healthy leaf. A brown line of greater or less width always sharply defines each lesion. (Fig. 1, A.).

Fructifications are produced in abundance on the upper surface of lesions that have attained the greyish stage and are apparent to the naked eye as minute black specks, frequently arranged in rows parallel with the midrib of the leaf. Lesions have not been observed on twigs.

<sup>1</sup> I learn on the authority of Prof. Guba that "The same fungus may be found in the Herbarium of Berlin Botanical Museum under the name of *P. funerea* Desm. *P. Podocarpi* Sacc., nom. nud. The fungus, however, has no relation to *P. funerea*." Under these circumstances the name *P. Podocarpi* is assigned to it.

<sup>2</sup> I desire to express my indebtedness to Prof. Guba for so freely placing his wide knowledge of this genus at my disposal.

<sup>3</sup> Guba, E. F. Monograph of the genus *Pestalotia* de Notaris. Part I. Phytopath. 19: 191-232. 1929.

<sup>4</sup> *Ibid.* Part II. Mycologia 24: 355-397. 1932.

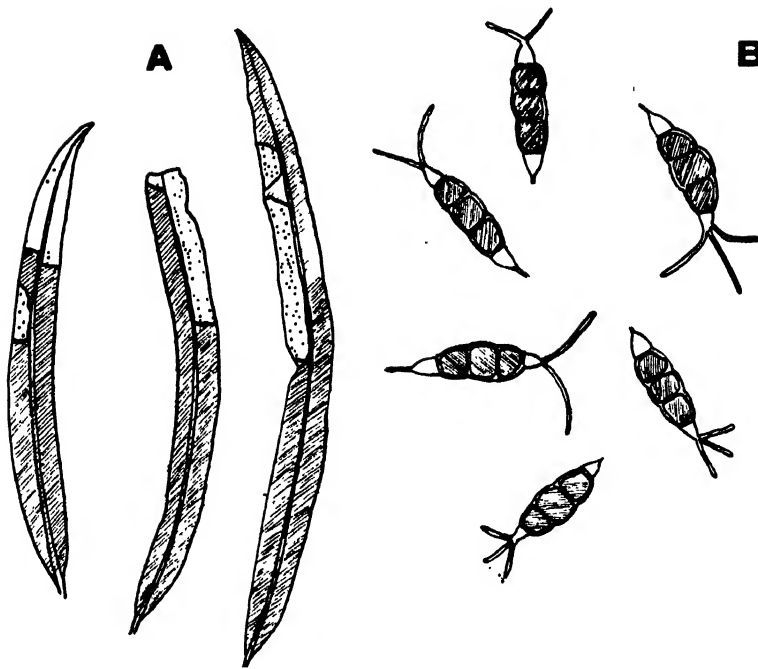


FIG. 1. A. Diseased leaves, showing acervuli. B. Spores of *Pestalotia podocarpi*.  $\times 850$ .

Owing to the thick, leathery character of *Podocarpus* leaves, pure cultures are readily obtained from fragments of diseased mesophyll or even more readily by the plating of spore suspensions. On 2 per cent malt agar an even, white, cottony growth quickly results at room temperature. Fructifications are produced in abundance as soon as the plate has become covered with mycelium. They tend to be somewhat larger than those developed on infected leaves and differ further in being well-formed pycnidia, having a definite ostiole from which a black spore coil is extruded. This condition has never been observed on the host, where spore production appears to be far less extensive than in pure culture, probably because of the more rapid exhaustion of the substratum. Spore dimensions, however, remain unchanged. No other form of fructification has been observed.

Cross sections of lesions revealed only mature acervuli. These develop between mesophyll and upper epidermis, the latter being lifted and finally ruptured. The fructification consists of a thin bed of hyphae spread over the upper surface of the mesophyll and a mass of spores filling the cavity. Close examination, however, reveals traces of a hyphal weft completely enclosing the fructification and providing a link with the well-developed pycnidia produced in pure culture. The latter arise as a submerged ball of

tightly interwoven hyphae, which becomes differentiated into an inner thin-walled mass surrounded by a zone 1-3 cells deep of thick-walled, brown hyphae. In the earliest stage observed the formation of a central cavity by the disintegration of the thin-walled, deeply staining tissue has already begun. It is followed by a great increase in volume of the young fructification, the result being a cavity lined and intersected by strands of disorganized material. Considerable tangential stretching is apparent in the cells of the pycnidial wall, from which it appears probable that the increase in bulk is a result rather of high osmotic pressure set up in the disorganized central mass than of rapid growth among the enclosing hyphae. A thick mass of sterile tissue remains at the upper surface of the developing pycnidium, and through this an ostiole is developed by mechanical stretching.

At this stage the uninucleate cells lining the central cavity become elongate and papillate, and form the sporogenous hyphae. In spore production a wall is first laid down delimiting the stalk cell. This is followed by one cutting off the apex. Two more walls then arise dividing the central area of the growing spore into 3 cells, which become brown and thick-walled when growth ceases. The appearance of the apical processes is the last phase in spore development.

Mature spores conform to the general *Pestalotia* type. (Fig. 1, B.). They are spindle-shape, frequently with one side more strongly curved than the other, and with markedly narrow extremities. The setae are simple, neither forked nor knobbed, and 2 or 3 in number. The dimensions quoted in the diagnosis are based on measurements of 100 mature spores from fructifications on foliar lesions.

**Diagnosis: *Pestalotia Podocarpi*, sp. nov.**

Acervuli epiphilli, innati, 200-240 × 130-170 μ atri, in maculis definitis, supra albidis, infra purpureo-brunneis; conidiis 5 cellularibus, anguste fusoides, utrinque fastigatis, rectis, vel nonnunquam leniter curvatis, vix ad septum constrictis, 17.5-24.5 μ loculis mediis olivaceis vel 2 superioribus leviter obscurioribus, 12-17.5 × 4.4-7.0 μ cellulis extremis turbinatis; ciliis saepe 3 raro 2, conidiophoris brevis.

Pustules epiphyllous, subepidermal, 200-240 × 130-170 μ, black, seated in definite spots, whitish above, purplish brown below.

Conidia 5-celled, narrow fusoid, tapering at both ends, straight or slightly curved, only slightly constricted at the septa, 17.5-24.5 μ. Median cells olivaceous or the upper 2 darker, 12-17.5 × 4.4-7.0 μ, end cells conic, setae usually 3 sometimes 2, pedicel short.

On living leaves of *Podocarpus elongata*.

Botanic Gardens, Glasgow, Scotland.

# INFLUENCE OF ROOTSTOCKS ON THE SUSCEPTIBILITY OF SWEET CHERRY TO THE BUCKSKIN DISEASE<sup>1</sup>

T. E. RAWLINS AND K. G. PARKER

(Accepted for publication November 7, 1933)

The symptoms of the buckskin disease of sweet cherry, *Prunus avium* L., were described in earlier publication (1). The most conspicuous symptoms are on the fruits, which are more or less conical in form, shrivel just before maturity, and have short pedicels. During the autumn months a peculiar red coloration is observed on the leaves along the base of the midrib and extending out along the lateral veins. Unlike many graft-infectious diseases the leaves of infected trees show no distinct yellowing or mottling during the growing season.

During an investigation made in 1928 it was observed that trees grafted on Mahaleb, *Prunus mahaleb* L., stock seldom carried fruit that showed symptoms of the disease. In 1929 a careful survey was made of an orchard in which some of the trees were on Mahaleb and some on Mazzard, *Prunus avium* L., stock. It was found that 52 of the 147 trees on Mazzard stock carried diseased fruit, while only 3 of the 82 trees on Mahaleb stock showed diseased fruit. The 3 affected trees on Mahaleb stock were each found to have a large sweet-cherry root growing from above the union, so that these trees were really partially on *P. avium* roots. These roots were cut from 2 of the diseased trees in order to determine whether the trees would recover when only Mahaleb roots remained. These trees have failed to show any evidence of recovery during the 3-year period since the removal of the sweet-cherry roots.

From these observations it appears that the Mahaleb root in some way causes a tree to resist or escape natural infection. On the other hand, even one *Prunus avium* root apparently confers susceptibility upon a tree. Growers in districts where the disease occurs are, therefore, using the Mahaleb stock in all new plantings.

Since very few trees in districts where the disease occurs are on Morello, *Prunus cerasus* L., stock no careful estimate has been made of the percentage of trees on this stock that have become naturally infected. However, such observations as have been made indicate that trees on this stock are very susceptible to the disease and, when infected, exhibit symptoms that are indistinguishable from those shown by diseased trees on Mazzard stock.

<sup>1</sup> Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

## GRAFTING EXPERIMENTS

Previous experiments (1) have indicated that the buckskin disease may be readily transmitted to healthy trees on Mazzard stock by infected Napoleon scions. More recently an attempt was made to infect young Napoleon trees on Mahaleb stock and others on Morello stock. The method of inoculation was the same as that described in a former paper<sup>2</sup> and consisted in grafting a diseased fruit-bud-bearing Napoleon scion on one branch of a young Napoleon tree and a healthy fruit-bud-bearing Napoleon scion on another branch of the same tree in order to insure fruit production and the possible detection of fruit symptoms. In 2 trees diseased buds were used instead of scions. The disease was found to be readily transmissible to Napoleon trees on Morello stock, 6 of the 7 grafted trees becoming infected. The fruit, leaf, and growth symptoms were similar to those exhibited by young diseased trees on Mazzard stock, namely, a decreased terminal growth, fruit conical in form, having short pedicels and shriveling just before maturity; as with infected trees on Mazzard stock no chlorosis of the leaves was observed.

The trees on Mahaleb stock gave very different results. Of the 8 trees grafted, 7 became very chlorotic and 2 had died within 7 months after grafting. Although 2 of the diseased fruit-bud-bearing scions used as inoculum produced typical diseased fruit, all of the fruit in other portions of the inoculated trees appeared normal. None of the 7 noninoculated check trees on Mahaleb stock showed any evidence of infection.

All of the above experiments have been carried out with young trees from 1 to 3 years old. On March 4, 1933, two large Black Tartarian trees (about 12 years old) having the union with Mahaleb stock at the ground level were each grafted with 10 diseased fruit-bud-bearing Napoleon scions. In one tree the scions were placed on small branches on the south side of the tree and in the other the 10 scions were distributed on small branches throughout the tree. The fruit on both the scions and other portions of the trees appeared to be normal when harvested in May and June, 1933. However, by September, 1933, the leaves on the scions were all extremely chlorotic and those on other portions of the 2 trees exhibited a chlorosis only slightly less severe than that of the scions. Two adjacent check trees of the same age remained normal.

It, therefore, appears that although trees on Mahaleb stock in some way escape or resist natural infection, they become very chlorotic after inoculation by grafting. This has been demonstrated for the Napoleon and Black Tartarian varieties.

<sup>2</sup> Rawlings, T. E., and W. T. Horne, "Buckskin," a destructive graft-infectious disease of the cherry. *Phytopath.* 21: 331-335. 1931.

All of the above-described work has been done on trees having the union with the stock near the surface of the ground. The question has arisen as to whether the causal agent of buckskin can pass from an infective Napoleon scion into a Mahaleb seedling and out into a healthy Napoleon scion placed on another branch of the Mahaleb seedling. Accordingly, 14 branched Mahaleb seedlings were planted in an experimental plot and an infected Napoleon scion was grafted on one branch and a healthy fruitbud-bearing Napoleon scion was grafted on another branch of 8 of the seedlings; 3 trees were grafted in 1932 and 5 in 1933. Six check trees were grafted to normal Napoleon scions only, in 1933. The grafting was always done in February or March. All of the diseased scions used as inoculum made little terminal growth, and the leaves on 7 of the 8 became very chlorotic, 2 of the 3 scions set in 1932 died before the second growth season. From the appearance of the 5 diseased scions set February, 1933, it is probable that most of these also will be dead before the 1934 growth season. None of these scions produced fruits.

Of the healthy scions placed on the 8 Mahaleb seedlings inoculated by grafting, all produced leaves which were normal in appearance and produced as much growth as the healthy scions placed on check trees. One of these scions produced 4 fruits which were normal in appearance.

The 6 check trees all remained normal in every respect. Two of the healthy scions placed on the check trees produced fruits that appeared normal. The above evidence, therefore, apparently indicates that the causal agent of buckskin does not readily pass through a Mahaleb stem.

#### SUMMARY

Sweet-cherry trees on Mahaleb stock in some way escape or resist natural infection with buckskin. However, trees on this stock become very chlorotic after grafting with diseased Napoleon scions. Such chlorotic trees rarely exhibit fruit symptoms, as contrasted to infected trees on Mazzard and Morello stocks that show little or no chlorosis and produce conical fruits that shrivel just before maturity and have abnormally short pedicels.

When diseased and normal scions were placed on different branches of Mahaleb seedlings the diseased scions became very chlorotic but the infection has not extended through the Mahaleb trees to the normal scions.

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## PHYTOPATHOLOGICAL NOTES

*A Disease of Banana, Markedly Similar to Bunchy Top, Produced by Celery Virus 1 in U. S. A.*—The discovery that banana (*Musa sapientum*) plants of the Lady Finger variety were susceptible to *Celery virus 1* was made on greenhouse-grown plants at Sanford, Florida. The important known virus trouble of this plant is called bunchy top and has been described by such workers as Magee<sup>1</sup> in Australia and Ocfemia<sup>2</sup> in the Philippine Islands. The disease has been considered by Smith<sup>3</sup> as a rosette type of trouble, inferred to belong to the yellows group of viruses. On certain bananas it produces rosette-like symptoms, and it has not been communicated to healthy plants by juice-inoculation methods.

In examining several thousand banana plants in the Sanford district, 6 were found with symptoms suggesting virus infection. These were destroyed by the owners. Natural occurrence of aphids on banana plants in Florida has never been observed by the writer. Medium-size "heads," or corms, were procured from healthy mats of plants in areas far removed from the places where apparently diseased plants occurred. These were planted in the greenhouse and in insect-proof cages. As soon as two leaves had developed they were subjected to experiment. The virus used in these studies has been described by the writer<sup>4</sup> and was obtained from cuttings of the same infected plant of *Commelina nudiflora*, from which was secured the original virus strain designated as *Celery virus 1*. One group of diseased shoots of *Commelina* was colonized for 14 days with *Aphis gossypii* and another group with *A. maidis*. From 75 to 150 such aphids were caged on banana leaves for inoculation. Of the 15 Lady Finger banana plants inoculated by *A. gossypii*, 13 became diseased, and both of the plants inoculated by *A. maidis* were infected. The incubation period varied from 21 to 33 days. Of the 7 plants held as controls, 4 were infested with virus-free aphids from healthy *Commelina*; none became diseased.

Early symptoms of celery-virus attack were usually first noticed on the third leaf appearing after inoculation. This leaf changed very little in size and general growth habit from the normal, though it was chlorotic, slow to unfurl, often drooped, and was severely marked with yellowish areas inter-

<sup>1</sup> Magee, C. J. P. Investigation on the bunchy top disease of the banana. Australian Counc. Sci. and Indust. Res. Bull. 30: 64 pp. 1927.

<sup>2</sup> Ocfemia, G. O. Bunchy-top of abacá or Manila hemp I. A study of the cause of the disease and its method of transmission. Amer. Jour. Bot. 17: 1-18. 1930.

<sup>3</sup> Smith, K. M. Recent advances in the study of plant viruses. 423 pp. Blakiston. Philadelphia. 1933.

<sup>4</sup> Wellman, F. L. Identification of *Celery virus 1*, the cause of southern celery mosaic. Phytopath. 24: 695-725. 1934.



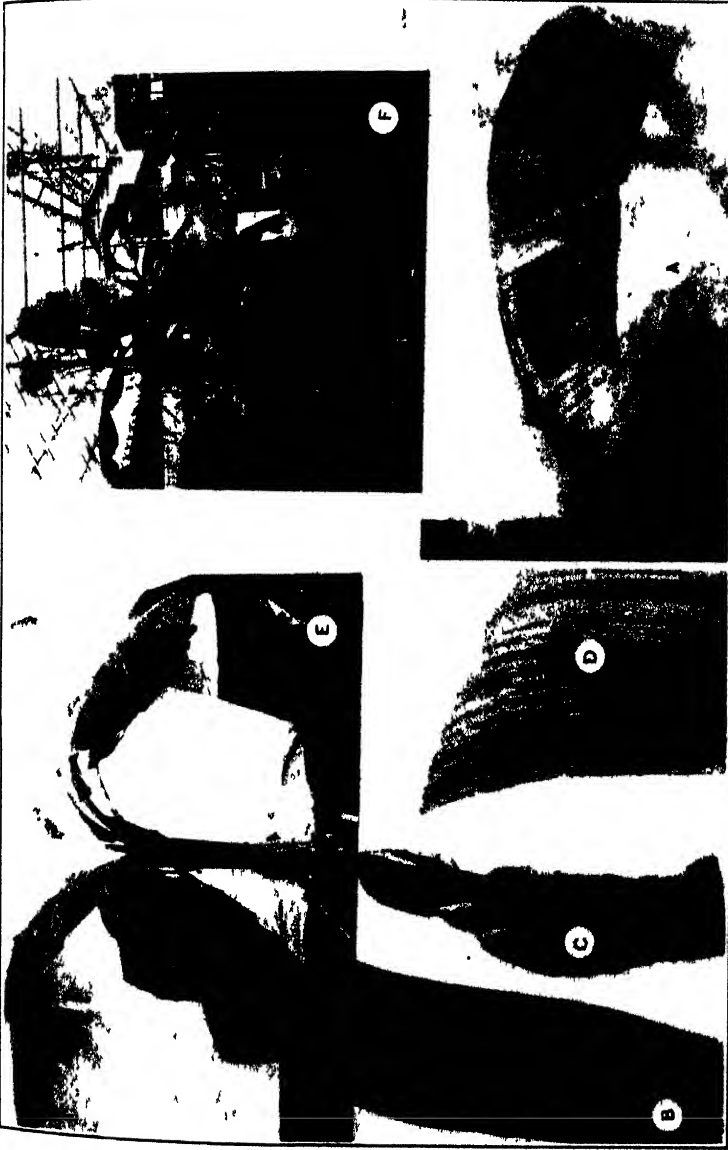


FIG. 1. Symptoms of *Celery virus 1* on Lady Finger banana. A. First leaf showing infection. Note darker stripes. B. Portion of one side of healthy leaf. C. Tip of the 7th diseased leaf from plant. Note stunting, malformation, and chlorotic streaks. D. Portion of one side of diseased leaf. Note light color, presence of necrotic streaks, and chlorotic streaks. E. Diseased plant, showing rosette appearance. F. Healthy plant (middle) and 2 diseased plants infected for 3 months. Note differences in height, leaf stunting, necrosis, malformation, and tendency of leaves to cup.

spersed with greener regions on the leaf blade (Fig. 1, A). Succeeding diseased leaves were tightly rolled (Fig. 1, E and F), and a healthy plant growing alongside produced 4 fully developed leaves, while 2 leaves on the diseased plant were pushing out. Diseased leaves were brittle, easily torn, had chlorotically spotted petioles with malformed fibrovascular bundles, and necrotic spots and streaks often occurred on the leaf lamina and pseudostem. There was distinct reduction of petiole and leaf blade and a consequent stunted rosette-like appearance (Fig. 1, E and F), like the bunchy-top symptoms described by Magee and Ocfemia referred to above.

When plants became diseased leaf sheaths were considerably reduced in rapidity of growth and flexibility. Partial strangulation of the newest leaves in the center of the pseudostem ended in their malformation (Fig. 1, E and C) and occasional splitting of the leaf sheaths. The leaves might be small but malformation did not occur if the pseudostem was slit with a knife. Two of the 9 healthy plants had started to produce fruit when experimentation was completed. Three other plants, when cut open, showed evidences of fruit-bud formation. No fruit buds were observed when 9 of the oldest severely diseased plants were dissected.

When aphids were transferred to the banana plants a few were put onto healthy *Commelina*, cucumber, and celery plants. These always produced typical symptoms of celery-virus infection. The celery virus was always easily recovered from the latter plants either by expressed juices rubbed on cucumber cotyledons (*cf.* Wellman<sup>5</sup>) or through transference by the use of *Aphis gossypii*. However, both methods were used with diseased bananas and it is curious that at no time was it possible to recover the virus from them. Juices from diseased and healthy banana leaves were highly toxic to celery and cucumber seedlings.

Magee's<sup>6</sup> description of bunchy top was confined mostly to its occurrence on Cavendish banana, *Musa cavendishii*, and 2 plants of this species were grown by the writer. Each was inoculated with 175 viruliferous *Aphis gossypii* and became diseased 30 days after inoculation. A week after unfurling, their leaves were slightly cupped, like those described and figured by Magee and Ocfemia. The leaf-cupping symptom was not strikingly present on Lady Finger plants. Diseased Cavendish plants were not maintained long enough to determine whether a rosetted appearance of the plant would result.—F. L. WELLMAN, Associate Pathologist, Bureau of Plant Industry, Washington, D. C.

<sup>5</sup> Wellman, F. L. *Loc. cit.*

<sup>6</sup> Magee, C. J. P. *Loc. cit.*

*Infection of Zea Mays and Various Other Gramineae by the Celery Virus in Florida.*—Studies of *Celery virus 1*, the cause of southern celery mosaic, have shown that the virus has a wide host range. Properties and description of the virus have been recently published by the writer.<sup>1</sup> Doolittle<sup>2</sup> was the first to find that the virus of celery mosaic occurred on the monocotyledonous host (*Commelina nudiflora*). The writer<sup>3</sup> showed while studying eradication measures for control of celery mosaic that around Sanford, Florida, *C. nudiflora* was undoubtedly one of the main reservoirs of the virus and the question of the susceptibility of other monocotyledonous plants naturally arose.

Plants of maize (*Zea mays*) were infected under greenhouse conditions in Florida by means of the viruliferous aphid, *Aphis gossypii*, from colonies on diseased *Commelina nudiflora* and celery. Hundreds of plants were inoculated and about two-thirds of them became diseased. Seedlings of Teosinté (*Euchlaena mexicana*), sweet-, milo-, and Kafir-sorghums (*Holcus sorghum*), wheat (*Triticum aestivum*), and rye (*Secale cereale*) were also successfully infected with *Celery virus 1*. In greenhouse studies sweet, pop, flint, and dent varieties of maize proved about equally susceptible. Several varieties of maize with typical symptoms of the celery virus attack also were found in abundance in fields in widely separate regions in Florida. Juices were obtained from diseased plants from greenhouse and field material, and they produced typical celery-virus lesions on cucumber cotyledons accompanied by systemic symptoms on cucumber and celery. In other properties the virus was identical with *Celery virus 1*.

Incubation periods of the disease on maize varied from 3 to 20 days. The most rapid and severe infection occurred at temperatures between 70° and 90° F., when 15 to 20 viruliferous aphids were placed on seedlings about 3 to 5 days after emergence above ground. Feeding was at first confined to the new, rapidly expanding, first seedling leaf. Primary lesions about 1 mm. in diameter appeared in 3 days around feeding punctures of viruliferous aphids. About 2 days later the lesions had spread downward along the veins of the seedling leaf (Fig. 1, A). Faint systemic symptoms occurred about 8 days after inoculation. Four days later systemic infection effects were distinct (Fig. 1, B). Striping and stunting were marked after the plants had been diseased from 3 to 4 weeks (Fig. 1, D).

The celery virus causes a stripe disease of maize in Florida very similar

<sup>1</sup> Wellman, F. L. Identification of *Celery virus 1*, the cause of southern celery mosaic. *Phytopath.* 24: 695-725. 1934.

<sup>2</sup> Doolittle, S. P. *Commelina nudiflora*, a monocotyledonous host of celery mosaic. (Abst.) *Phytopath.* 21: 114-115. 1931.

<sup>3</sup> Wellman, F. L. Celery mosaic control in Florida by eradication of the wild host *Commelina nudiflora*. *Science n.s.* 76: 390-391. 1932.

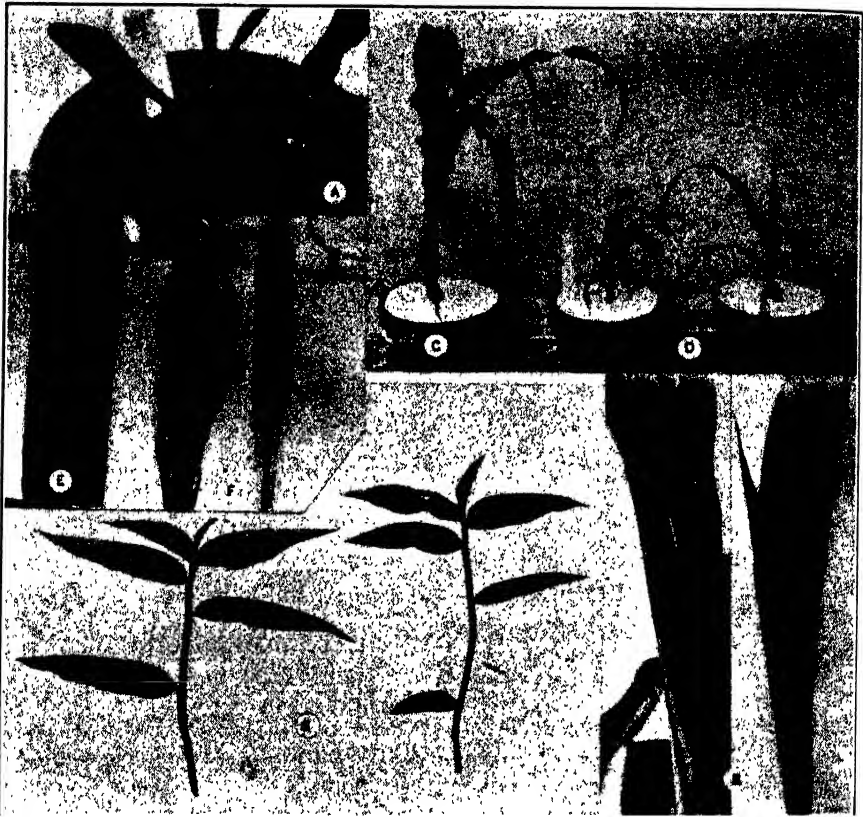


FIG. 1. Symptoms of *Celery virus 1* on maize and *Commelina nudiflora*. A. Primary lesions on first cotyledonary leaf of maize seedlings, 5 days after being infected by viruliferous *Aphis gossypii*. Note spread of lesions downward along veins. B. Early systemic infection symptoms 12 days after infection. C. Healthy maize plant about one-third mature height. D. Maize plants of same age as C but severely diseased with celery virus stripe. E. Portion of leaf from healthy maize plant of same age as leaves in F. F. Celery virus striped leaves from 2 plants infected nearly 6 weeks. Note splitting of lamina, crumpling of tip, and severe stunting. G. Shoots of *Commelina* severely diseased with *Celery virus 1*. Plants with symptoms of this type were used as the source of virus. Compare these chlorotic patterns with symptom expressions illustrated in B, D, and F.

to but not identical with the white stripe of maize in Cuba. Virus diseases of maize similar to Cuban white stripe described by Stahl<sup>4</sup> are widely distributed in the tropics as is indicated by reports from Hawaii, Africa, Cuba, and Trinidad. The writer visited Cuba recently and found typical symptoms of infection by *Celery virus 1* on celery, squash, cucumber,

<sup>4</sup> Stahl, C. F. Corn stripe disease in Cuba not identical with sugar cane mosaic. Trop. Plant. Res. Foundation. Bull. 7. 1927.

*Commelina nudiflora*, *C. communis*, pepper, sweet potato, and tomato. Maize, in fields planted at a later time than the vegetables, was diseased with typical white stripe but not with celery virus.

After numerous experiments it was evident that the celery-virus stripe on maize in Florida is transmitted by aphids but not by *Peregrinus maidis*, which Stahl<sup>4</sup> found was the vector of Cuban white stripe. Symptoms on celery-virus diseased plants begin about insect punctures followed by development of round primary lesions that spread downwards (Fig. 1, A). Early evidence of systemic infection consist of numerous light colored, intermittent, elliptical spots of various lengths and widths whose long axes are parallel with veins of the leaves (Fig. 1, B). As infection becomes severe, plants are badly stunted (Fig. 1, D) and are remarkably similar to white stripe-infected plants. They lack, however, the severe rosette and crook-neck symptoms of that disease. Chlorotic patterns often occur similar to cane mosaic on maize (cf. Brandes<sup>5</sup>). Occasional plants affected with the celery-virus-stripe have darkened fibrovascular bundles in the stalks similar to Kunkel's<sup>6</sup> description of the Hawaiian virus disease of maize. Well-advanced celery-virus striping of leaves is more translucent than in Cuban white stripe, with distinct demarcation between chlorotic and green areas. There is a tendency to leaf splitting (Fig. 1, F) and sometimes crumpling of leaf tips. Moiré mottling is found on the first systemically infected leaves, and buff colored and necrotic areas on old severely affected leaves.—F. L. WELLMAN, Associate Pathologist, Bureau of Plant Industry, Washington, D. C.

*The Production of Perithecia in Ceratostomella ips Rumb.*<sup>1</sup>—In the course of a study of the interrelation of bark beetles and blue-staining fungi<sup>2</sup> a number of single-ascopore cultures of *Ceratostomella ips* Rumb. were isolated. It was observed that some of the cultures produced both perithecia and conidia, while others produced conidia only. This led to further experiments. The results obtained so far are so different from those reported by other investigators working with other species of *Ceratostomella* that it seems desirable to place them on record.

Dade<sup>3</sup>, in 1928, was the first to demonstrate heterothallism in the genus

<sup>5</sup> Brandes, E. W. Mosaic disease of corn. Jour. Agr. Res. 19: 517-522. 1920.

<sup>6</sup> Kunkel, L. O. A possible causative agent for the mosaic disease of corn. Bull. Exp. Sta. Hawaiian Sugar Plant. Assoc. Bot. Ser. 3: 44-58. 1921.

<sup>1</sup> Paper No. 1265 of the Journal Series of the Minnesota Agricultural Experiment Station.

<sup>2</sup> Leach, J. G., L. W. Orr, and Clyde Christensen. The interrelation of insects and fungi in the deterioration of felled timber: I. Bark beetles and blue-staining fungi in Norway pine. (In press.)

<sup>3</sup> Dade, H. A. *Ceratostomella paradox*, the perfect stage of *Thielaviopsis paradoxa* (de Seyne) von Höhnelt. Trans. Brit. Mycol. Soc. 13: 184-194. 1928.

*Ceratostomella*. He demonstrated the existence of “+ and -” strains in *C. paradoxa* (de Seynes) Dade. In 1932 Gregor<sup>4</sup> showed that *C. pluriannulata* Hedg. is heterothallic. In the same year Mittmann<sup>5</sup> reported evidence showing that *C. fimbriata* (E. and H.) Elliot and *C. quercus* Georg. are homothallic and that *C. coerulea* Münch. is heterothallic.

Buisman<sup>6</sup> has shown recently that single-ascospore cultures of *Ceratostomella fagi* Loos would produce perithecia, but that cultures from single conidia failed to produce them. However, when 8 single-conidium cultures were mated in the usual manner 2 sexually different groups were discovered, each of which produced perithecia when mated with a culture of the opposite sex. This phenomenon was explained by assuming that segregation for sex was delayed until after the germination of the ascospores and was complete only when the conidia were formed.

In the work here reported the single spores were picked up with a Chamber's micromanipulator and allowed to germinate under observation on a hanging drop of agar after which they were transferred to individual tubes of agar for further observation. Figure 1 shows in diagram the pedigree of the cultures used in the study of perithecial production in *Ceratostomella ips*. It will be noted that of a total of 31 single-ascospore cultures 20 produced perithecia and 11 produced conidia only. From a total of 32 single-conidium cultures from perithecia-producing colonies only 10 produced perithecia. None of the 18 single-conidium cultures, from cultures producing only conidia, formed perithecia. These results cannot be interpreted to indicate heterothallism with delayed segregation for sex, because, when the apparently haploid cultures were mated with each other (See figure 1 for cultures mated), no perithecia were produced. In all cases the fungi were grown on 1 per cent malt-extract agar and control cultures of perithecia-forming strains were included in order to be sure that the medium was suitable for perithecial production. In several cases duplicate cultures were made on other media, but the results were always the same.

As a further check 3 groups of cultures were grown together in Petri dishes on 4.5 per cent malt-extract agar, being planted around the periphery of the dishes as follows: Dish 1—a, 1, b, 2, c, 3; Dish 2—k, 6, l, 7, d, 5; Dish 3—3, 10, f, 11, g, 9; but no perithecia were produced.

In addition, many of the cultures, singly or in combination with others,

<sup>4</sup> Gregor, Mary J. F. A study of heterothallism in *Ceratostomella pluriannulata*, Hedgecock. Ann. Mycol. 30: 1-9. 1932.

<sup>5</sup> Mittmann, Gertrude. Kulturversuche mit Einsporstämmen und zytologische Untersuchungen in der Gattung *Ceratostomella*. Jahrb. Wiss. Bot. 77: 185-219. 1932.

<sup>6</sup> Buisman, Christine. Über die Biologie und den Parasitismus der Gattung *Ceratostomella* Sacc. Phytopath. Zeitschr. 6: 429-440. 1933.

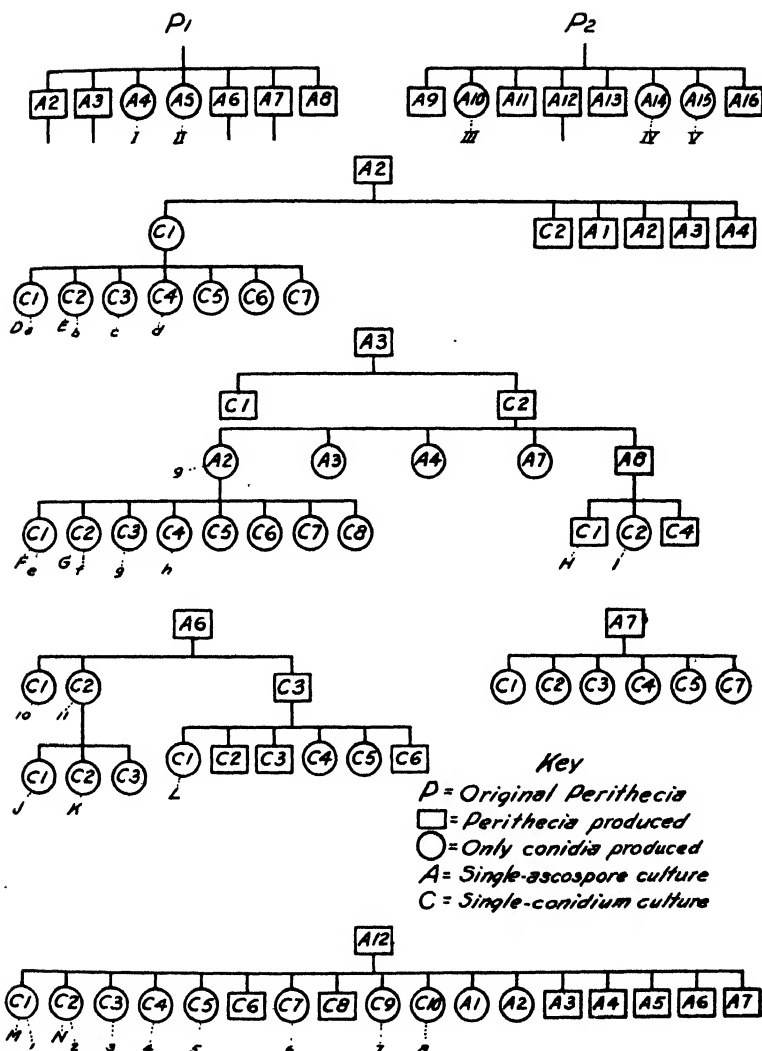


FIG. 1. Diagram showing the pedigree of single-spore cultures of *Ceratostomella ips* and indicating the kinds of spores formed by each culture. Four different groups of cultures were mated in all possible combinations within each group. These groups are labelled as follows: Group 1, I to V; Group 2, a to h; Group 3, 1 to 8; and Group 4, D to N.

were subjected to ultraviolet light of various intensities and durations without stimulating the formation of perithecia.

From the results obtained by mating, it would appear that the loss of the ability to produce perithecia was due to something other than simple segregation for sex. Of course, it is entirely possible that, if a sufficiently

large number of cultures were mated, the proper combination would be found, but, from the experience of various investigators with heterothallic fungi, it would seem that the matings reported here would have been sufficient to bring together at least some of the complementary sex groups.—J. G. LEACH, University Farm, St. Paul, Minn.

*Types of Mosaic on Sugar Cane in Louisiana.*<sup>1</sup>—Many variations and types of sugar-cane mosaic symptoms have been described and illustrated in the literature. To the writer's knowledge proof has been lacking that such represented other than differences ascribable to varietal reaction or environmental conditions influencing the manifestations of a single causative agent. However, Yoder<sup>2</sup> and Tims and Edgerton<sup>3</sup> have noted unusual behavior of the disease that suggested a difference in cause, although definite separation of strains was not reported. In 1932, the writer found two widely divergent types of mosaic symptoms, (1) mild and (2) severe, occurring naturally on adjacent stools of a single seedling variety, C. P. 28/60, in the nursery plantings of the United States Department of Agriculture at Houma, La. In 1933, 2 additional types (Nos. 3 and 4) were collected in commercial fields of Co. 281. All 4 types have persisted without apparent change through successive vegetative propagations of the infected plants.

In order to determine whether these 4 types represent merely differences in host expression or actual differences in the causative agent, or agents, 4 inoculation experiments employing the needle-prick method were conducted in an insect-proof greenhouse. Usually a high percentage of disease transmission was obtained. Parallel controls, without exception, remained symptom-free. The results may be briefly summarized as follows: The 4 distinct mosaic patterns were readily perpetuated on the original differential varieties (C. P. 28/60 and Co. 281) by transfer of inoculum from diseased to healthy plants of the same variety. All types were then inoculated separately into parallel groups of healthy plants of Co. 281, Louisiana Purple, and the P. O. J. numbers 36-M, 213, and 234. On these varieties types 1, 2, and 4 were not differentiated but gave only indistinguishable mosaic symptoms that were typical of the disease as it occurs on each variety in commercial fields. Type 3, however, reproduced on all these varieties its distinctive yellowish-white stripes that had not previously been encountered except on occasional collections of Co. 281. Inoculum extracted separately

<sup>1</sup> The writer is indebted to Dr. R. D. Rands for valuable advice during the progress of this study and for making the photograph reproduced in figure 1.

<sup>2</sup> Yoder, P. A. Rare cases of mosaic disease in highly resistant varieties of sugar cane. U. S. Dept. Agr. Dept. Circ. 392. 1926.

<sup>3</sup> Tims, E. C., and C. W. Edgerton. Behavior of mosaic in certain sugar-cane varieties in Louisiana. (Abst.) Phytopath. 22: 27. 1932.



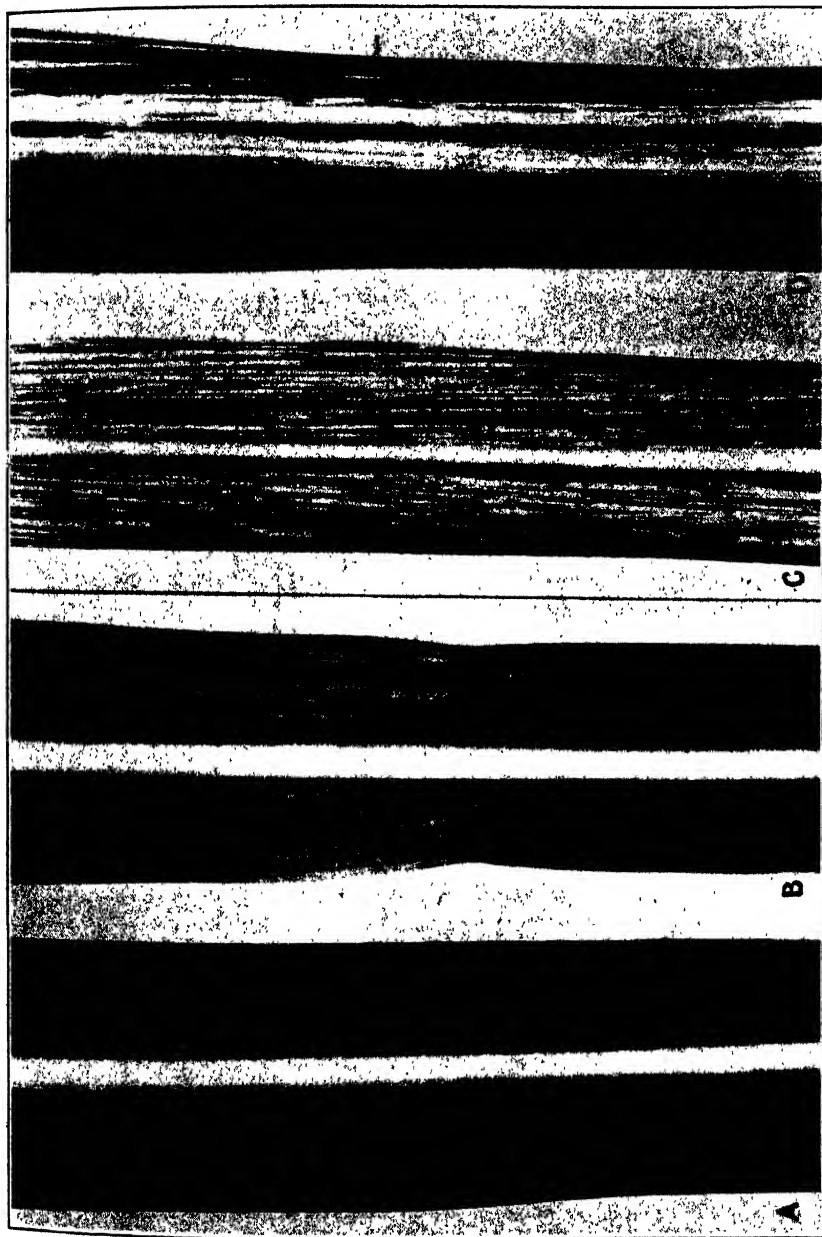


FIG. 1. Three types of mosaic symptoms on leaves of the sugar cane variety C. P. 28/60. A. Healthy; B. Mosaic type No. 1. C. Type No. 2. D. Type No. 3.

from the 3 groups of Louisiana Purple (and later other varieties) showing identical symptoms as well as from the type-3 plants and introduced into healthy groups of C. P. 28/60 and Louisiana Purple invariably reproduced in typical pattern types 1, 2, and 3 on C. P. 28/60 (Fig. 1). Type 4, while indistinguishable from type 3 on this variety, invariably produced the ordinary pattern on Louisiana Purple in contrast with the severe symptoms of type 3. Preliminary tests with inoculum mixtures failed to reproduce type 3 from any combination of the remaining types.

From the consistency with which the 4 types of symptoms have been reproduced in these experiments, a difference in causative agent, or agents, would seem to be indicated as nearly as can be predicted on symptomatology. Possibly these types are the expression of individual strains or temporary modifications of a single virus, or they may represent distinct virus diseases. In either case it is recognized that they may be further resolved by the discovery of additional differentiating hosts. The types may be tentatively described as they occur on 1 to 4-months-old plants, as follows:

*Type 1* is characterized by a slight mottling with very little chlorosis and no noticeable stunting of C. P. 28/60 (Fig. 1, B) and by the production of ordinary (typical for the variety) mosaic symptoms on Louisiana Purple, Co. 281, P. O. J. 36-M, P. O. J. 213, and P. O. J. 234.

*Type 2* appears as a severe mottling with large chlorotic areas, a varying extent of necrosis, and marked stunting of C. P. 28/60 (Fig. 1, C) and only ordinary mosaic symptoms as in type 1 on the other varieties.

*Type 3* first becomes evident on C. P. 28/60 by the development of elongated, almost white blotches or islands, some of which later coalesce into long, yellowish white streaks, often running the full length of the older leaves (Fig. 1, D). The streaks may follow the midrib, and are frequently accompanied by necrosis, sometimes so severe as to produce temporary blighting, and sometimes death, of the growing point. Severe symptoms similar to the above appear also on Louisiana Purple and the four other varieties.

*Type 4* on C. P. 28/60 is identical in appearance with type 3, but in common with types 1 and 2, manifests only ordinary mosaic symptoms on the five other varieties.

Thus far, type 1 has been obtained only from 4 C. P. seedlings; types 2 and 4 quite frequently from Co. 281, and type 4 from all specimens of P. O. J. 213 thus far collected and tested. Type 3 is apparently very limited in distribution. However, more extensive surveys of commercial varieties and fields, and inoculation tests now in progress should throw more light on type distribution, varietal susceptibility, and economic importance as well as the phenomenon of recovery on which many conflicting (unpublished) data have been accumulated.—EATON M. SUMMERS, Bureau of Plant Industry, Houma, Louisiana.

*Panicles of Rexoro Rice Injured at Emergence by Sun Scald.*—During the latter part of August, 1932, a characteristic blighting of panicles of Rexoro rice in some fields south of Lake Charles, Louisiana, was called to the attention of the senior author by C. C. Collett, the local county agent. In a certain field 5 to 10 per cent of the plants scattered throughout the area were severely injured, and the farmers in the vicinity feared that some new disease was attacking their rice. This severely attacked field was visited again on September 7 and 13. The blighting occurred to some extent over this entire period of about three weeks.

It was distinctly different from "straighthead" in that the affected panicles or portions of panicles were bleached and killed as if scalded. In some cases only the upper portions of certain spikelets showed injury, and the lower portions of the spikelets and panicles apparently had been protected, when the injury occurred, by the upper parts of the leaf sheath. Some panicles showed injury only in the upper or middle portions, while others were completely blighted. The badly blighted panicles remained erect, as in straighthead, while the uninjured panicles turned down as they began to fill.

The most recently blighted spikelets were noted on panicles that had emerged within one or two days. These spikelets still retained some green color, those that had been blighted for a slightly longer time were bleached nearly white, and those that had been blighted for a still longer time were overgrown to a greater or lesser extent by saprophytic black molds. On the basis of the relative abundance of the black molds, particularly at the time of the last visit, it was possible to estimate the approximate time when the various panicles had been injured.

Through the courtesy of Mr. Collett, the weather records for Lake Charles for 1932 and the preceding 3 years were secured. It was found that during the 3-week period in 1932, in which blighting of Rexoro occurred, higher temperatures and greater wind velocities had been recorded than in the same period of the preceding 3 years. Undoubtedly these high temperatures and wind velocities, together with bright sunshine, produced the scald injury. The panicles of Rexoro appear to be very delicate as they emerge from the boot and, therefore, seem especially susceptible to this type of injury. J. Mitchell Jenkins states that once or twice sun scald has been observed on Rexoro panicles at the Rice Experiment Station, Crowley, La.

A somewhat similar injury was reported by Tisdale and Jenkins<sup>1</sup>, both for California and Louisiana, on varieties other than Rexoro. However, the injury in California apparently was somewhat different, as it was "more noticeable on levees and on land where there is a lack of water,"

<sup>1</sup> Tisdale, W. H. and J. M. Jenkins. Straighthead of rice and its control. U. S. Dept. Agr. Farmers' Bull. 1212. 1921.

whereas the injury to Rexoro occurred on plants scattered throughout normally submerged portions of the field and apparently was no more abundant on the levees than elsewhere.

After September 13 no further injury was noted by either Mr. Collett or the farmer in the field of Rexoro under observation. While some reduction in yield resulted, a fairly satisfactory crop was obtained.—E. C. TULLIS, A. L. SMITH, AND A. G. JOHNSON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

*The Inability of Aplanobacter Insidiosum to Enter Alfalfa Seedlings in the Absence of Wounds.*<sup>1</sup>—It has been generally accepted that the entrance of the wilt organism, *Aplanobacter insidiosum* L. McC., into alfalfa is effected only through freshly made wounds of any type or location, on either the above- or below-ground plant parts. To determine whether the bacteria are capable of entering any natural portal the following experiment was undertaken in the fall of 1932. A greenhouse bench, 40 × 3 ft., was so filled with composted sod soil that from the center to either end of the bench there was a drop of about 4 inches in the soil level. The bench was divided into 20 blocks 2 × 3 ft. in area.

Eight hundred one-month-old seedlings of Grimm alfalfa were inoculated by severing the ends of the roots under a bacterial suspension and planted in the 4 center blocks at the crest of the soil slope. Each successive block to either end of the bench was then planted to noninoculated seedlings at the rate of 200, 150, 120, 100, 80, 60, 40, and 32 plants. Approximately 2500 plants were employed.

After 5 weeks, when symptoms were first noted in the inoculated area, all the watering for the bench was done from the center blocks, the slope of the soil being sufficient to carry the water rapidly to either end of the bench. As the plants began to die off in the infected area, the force of the water was strong enough to distribute the dead plant parts over the entire bench. The top growth was cut once during the course of the experiment with due precautions to avoid any infection of the noninoculated plants. Water was kept away from the plants until the wounds made by cutting the tops were closed.

At the end of 5 months a detailed examination of all individuals was made for evidences of wilt infection. Of the 800 plants inoculated only 5 per cent were found healthy and 20 per cent were slightly infected, whereas the remainder were moderately to severely diseased or dead. In no instance was even a slightly infected plant found in any other part of the bench, irrespective of the rate of planting. It can be concluded that the bacteria, at least under the conditions of the above experiment, are unable to enter

<sup>1</sup> Journal Series Paper No. 151, of the Nebraska Agricultural Experiment Station.

alfalfa plants in the absence of freshly made wounds. It may also be safe to assume that the organism causing bacterial wilt is unable to enter any natural portal on older alfalfa plants under ordinary field conditions.—GEORGE L. PELTIER, Nebraska Agricultural Experiment Station, Lincoln, Nebr.

*The Effect of Mildew Infection on the Response of Wheat-Leaf Tissues Normally Resistant to Leaf Rust.*<sup>1</sup>—The effect of infection by one fungus upon the response of the host plant of another fungus occasionally has been mentioned in phytopathological literature. A striking demonstration of the breaking down of the resistance to leaf rust of the leaf tissues of certain wheat varieties by earlier infection by mildew has been observed repeatedly at Manhattan, Kansas, in greenhouse experiments with *Puccinia triticina* Eriks. The phenomenon in these cases has consisted in the development of localized areas of susceptible tissue in leaves, normally resistant to leaf rust, after they had been attacked by mildew.

Early in the leaf-rust studies the writer noted that occasional large, normal uredia developed on wheat plants resistant to the physiologic form with which they were inoculated<sup>2</sup>. These were at first regarded as chance mixtures of some other form, but reisolation cultures proved this not to be the case. Such cultures, when used to inoculate seedlings of the variety upon which the uredia had developed, produced only the typical resistant type of reaction. Tests with differential varieties proved them to be pure cultures of the form originally used as inoculum.

A closer examination of many such uredia revealed that they nearly always were closely associated with infections of mildew, *Erysiphe graminis* DC. Uredia developed embedded directly in the mycelial web of the mildew, in the narrow chlorotic area immediately surrounding the mycelial web, or in the chlorotic area on the surface of the leaf opposite a mildew spot.

The effect of mildew infection on the response to leaf rust of a plant of the variety Warden (C.I. 4994) is shown in figure 1. This plant was grown in the greenhouse in 1931 and was inoculated with a pure culture of leaf rust form 9. The mildew developed from natural infections. It will be noted that several large uredia have developed in the mildewed areas, while outside of such areas leaf-rust infection is manifest only as

<sup>1</sup> Contribution No. 341 from the Department of Botany, Kansas Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, cooperating.

<sup>2</sup> EDITOR'S NOTE: Following the acceptance of the manuscript for this note it was discovered that the same phenomenon is discussed in a manuscript by E. B. Mains, submitted for publication in PHYTOPATHOLOGY on Jan. 10 and accepted on Jan. 17, 1934.

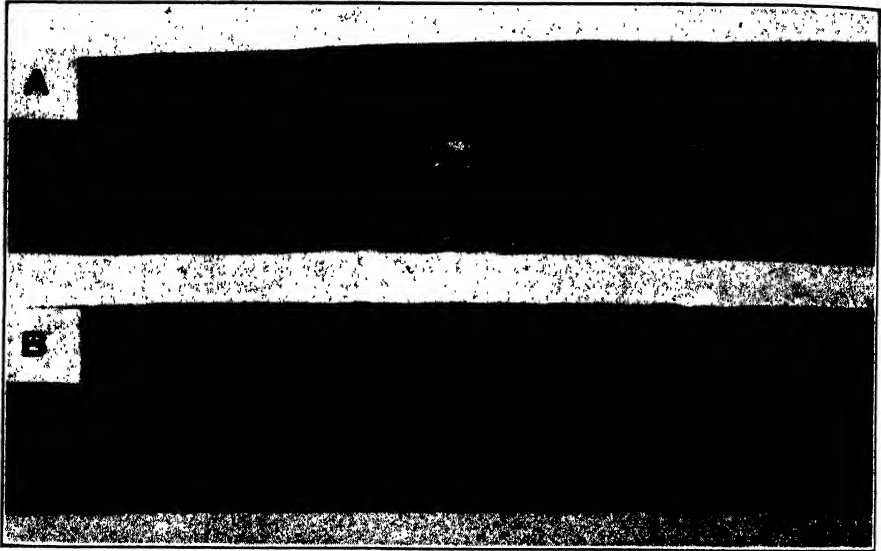


FIG. 1. Sections of a leaf of Warden (C. I. 4994) spring wheat affected by mildew and leaf rust. A. Lower surface showing uredia of leaf rust in the mycelial wefts of mildew. B. Upper surface of the same leaf showing leaf-rust uredia in chlorotic areas on the leaf surface opposite a mildew spot. Small chlorotic flecks caused by leaf-rust infection in mildew-free tissue appear in both A and B.

numerous sterile flecks. A section of the lower surface of the leaf with uredia imbedded in several of the mildew mats is shown at A, while B shows a section of the upper surface of the same leaf with uredia in a chlorotic spot resulting from mildew on the opposite surface.

Warden is consistently highly resistant to physiologic form 9, with which this plant was inoculated. Flecking, but no uredia, usually result from such inoculation. Isolations from several uredia on this plant gave only pure form 9, which in turn produced only flecking on mildew-free seedlings of Warden.

No explanation is available for the changing by mildew of localized areas of leaf tissue from a resistant to a susceptible condition with regard to leaf rust. Since spots affected by mildew usually are accompanied by chlorosis, it would seem obvious that some change has occurred in the leaf tissues. The fact that leaf rust develops normally on such tissues suggests that these changes are of a chemical nature. It seems probable that the mildew fungus splits some complex compounds, which the rust fungus is unable to use, into simpler compounds upon which it is able to subsist.—C. O. JOHNSTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Kansas Agricultural Experiment Station.

*The Occurrence of Stem Rot of Rice in California.*—The rice crop in California has been exceptionally free of disease. Therefore, finding stem rot (*Leptosphaeria salvinii* Catt.) there in 1932 was of considerable interest. In the spring of 1932, seed of Early Prolific (known also as Early Blue Rose), obtained from the Woodland Rice Mill, Woodland, Yolo County, Calif., was sown in the replicated varietal plots at the Biggs Rice Field Station. In 1931, this mill had grown a small acreage of Early Prolific in Yolo County from seed obtained from Arkansas that year, and apparently the seed sown at Biggs in 1932 came from that crop.

In examining the rice varieties at the Station in September, 1932, a plant of Early Prolific was found to be infected with stem rot in the conidial and sclerotial stages. No other infected plants were found.

In the fall of 1933 stem rot was observed on 6 varieties in the Station plots and on Early Prolific in a commercial field in Yolo County, but apparently it had done no serious damage. It is, therefore, probable that there were other infected plants present in 1932 that were overlooked.

Owing to the general distribution of the disease in Arkansas, it is probable that the conidia and sclerotia of this fungus are carried on seed rice. Since stem rot was first found in California on an Early Prolific plant, it seems very likely that the disease was introduced with seed of that variety in 1931.—E. C. TULLIS, JENKIN W. JONES, AND L. L. DAVIS, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the California Agricultural Experiment Station.

# THE CROP PROTECTION INSTITUTE

## SUMMARY OF PROGRESS

January 1, 1934

The following is a condensed summary of the progress and work of the Crop Protection Institute from the time of its organization, 12 years ago, to the first of the current year. It has been prepared in order to give a general view of the institute's operations and of the results accomplished and to summarize briefly the method that it follows in all of its research projects.

From the beginning all of the institute's activities have been controlled by a Board of Governors of 9 men. Three members of this board are chosen by The American Phytopathological Society, one being named each year for a 3-year term. Three members are chosen similarly by the American Association of Economic Entomologists. Two are named by the Association of Official Agricultural Chemists. One is designated by the National Research Council.

The board elects its own chairman who serves as administrative officer in financial matters, and is *ex officio* a member of the institute's various technical committees in order to perform necessary administrative functions. Technical direction of the research projects, however, lies with technical committees representing the board. The board elects a secretary-treasurer who for many years has been Mr. Paul Moore, who has his office in the building of the National Research Council, in Washington. The financial depository of the institute also is in Washington.

Recently, at the suggestion of the chairman of the board, provision was made for election of a vice-chairman, who would work with the chairman and help him in various contacts with industrial organizations.

Under the institute's invariable procedure every research project or development undertaking is maintained as a separate entity. It has its own technical committee who plan the project in detail, review the progress of it, select the investigator or investigators employed on the project, and examine and interpret the results.

Initial contacts with various industries that have possible research to undertake are made by the chairman of the board with the help of the vice chairman. These contacts are necessarily many and varied. Some of them are made because inquiries are addressed to the institute. This, of course, occurs to an increasing degree as the work of the institute becomes more and more wide-known. Other contacts are made because the general nature of a given industry, or the nature of products made by it, suggest that



interesting research could be undertaken. This is especially true in the chemical industry, where invariably there are various by-products, some of which have never been explored as to their application to horticulture, agriculture, animal industry, or other related activities. Such contacts as these have been made with several hundred representatives of the industrial world. Out of these has come the institute's research program during the past 12 years.

If a contact develops the possibility of a research project, the matter is immediately submitted to proper technical experts on the board of governors and frequently to additional experts from among the scientific membership of the institute. Through such consultation the initial study of a possible project is made, tentative plans are drawn up, and a tentative budget is worked out. If the entire matter looks sound and sufficiently interesting scientifically, it is submitted in time to the board of governors of the institute as a proposed project, and the board votes whether the institute shall or shall not enter into an agreement to carry on the projected research.

If a decision is made to enter upon the new project, a technical committee is named immediately and this committee, as already indicated, represents the board in handling all details of the project, including the selection of a man, or men, to be employed and the layout of the research program. Under the institute's practice the committee, although fully responsible for the project, works in close harmony with the technical staff of the company that is providing funds for the new project. Through this close cooperation results are secured that would not otherwise be possible, while at the same time the institute retains full authority and responsibility and thus safeguards the scientific status of its work.

The following is a brief summary of research projects undertaken to date. Within the space available only the title of a project and brief facts as to research men employed on it and the resultant publications can here be given. Full technical reports on many of these research projects will be found in the institute's bulletin series.

*Sulphur Investigations.*—Supported jointly by the Freeport Sulphur Company, the Texas Gulf Sulphur Company and the Union Sulphur Company. Headquarters: the Missouri Botanical Garden, the Geneva, N. Y., Agricultural Experiment Station, and the Boyce Thompson Institute. Investigators, H. C. Young, A. Hartzell, F. H. Lathrop, L. Tisdale. Five publications, Bulletins Nos. 3, 5, 6, 7, and 8.

*Scalecide Investigations.*—Supported by the B. G. Pratt Company. Headquarters: Pennsylvania Agricultural Experiment Station, State College, Pa. Investigator, John W. Miller.

*Furfural Investigations.*—Supported by the Quaker Oats Company. Head-

quarters: Iowa State College, Ames, Iowa. Investigators, H. H. Flor and C. S. Reddy. Publications, Bulletins Nos. 13 and 24.

*Crown Gall Project*.—Supported by U. S. Department of Agriculture, American Association of Nurserymen, Iowa State College, University of Wisconsin, and Boyce Thompson Institute. Headquarters: Iowa State College, Boyce Thompson Institute, and University of Wisconsin. Investigators, M. K. Patel, A. J. Riker, J. H. Muncie, and W. B. Shippy. Twelve publications, Bulletins Nos. 9, 11, 16, 18, 19, 20, 21, 25, 26, 27, 29, and 39.

*Copper Studies*.—Supported jointly by Nichols Copper Company and Goldsmith Bros. Smelting and Refining Company. Headquarters: Boyce Thompson Institute. Investigator, Frank Wilcoxon.

*Standard Oil Emulsion Project*.—Supported by the Standard Oil Co. (Indiana). Headquarters: the Illinois Agricultural Experiment Station, Urbana, Ill. Publications, Bulletin No. 33.

*Flit Investigation*.—Supported by the Standard Oil Company of New Jersey. Headquarters: New Jersey Agricultural Experiment Station. Investigator, F. C. Nelson. Publication, Bulletin No. 12.

*Kip and Bovinol Project*.—Supported by Standard Oil Co. (Indiana). Headquarters: Purdue University Agricultural Experiment Station. Investigator, H. G. Butler.

*Bayer Company Project*.—Supported by Bayer Company, Inc. Headquarters: the Boyce Thompson Institute. Investigator, J. L. Horsfall. Publication, Bulletin No. 15.

*Thallium and Mercury Project*.—Supported by Bayer Company, Inc. Headquarters: Boyce Thompson Institute. Investigator, C. R. Orton.

*Airfloat Project*.—Supported by Standard Oil Company of New Jersey. Headquarters: New Jersey Agricultural Experiment Station. Investigator, R. C. Burdette.

*Horticultural Sprays Project*.—Supported by Standard Oil Company of New Jersey. Headquarters: Connecticut (State) Agricultural Experiment Station. Investigator, Neely Turner. Publication, Bulletin No. 38.

*Pyrethrum Project*.—Supported by Standard Oil Company of New Jersey. Headquarters: New Jersey Agricultural Experiment Station; Lexington, Kentucky; Florida Experiment Station; and Pennsylvania. Investigator, R. E. Culbertson.

*Phinotas Investigation*.—Supported by Phinotas Chemical Company. Headquarters: New Hampshire Agricultural Experiment Station. Investigator, M. Stewart. Publication, Bulletin No. 14.

*Flotation Sulphur Project*.—Supported by Koppers Products Company. Headquarters: University of Illinois. Investigators, M. A. Smith, J. M. Hamilton, G. H. Richter, and C. E. Baker. Publications, Bulletins Nos. 28 and 33.

*Oxidized Oils Project*.—Supported by Kay Laboratories, Inc. Headquarters: Maryland Agricultural Experiment Station. Investigator, J. L. Hoerner. Publication, Bulletin No. 23.

*Shale Oils Project*.—Supported by Tennessee Copper & Chemical Company. Headquarters: Ohio Agricultural Experiment Station and at Uvalde, Texas. Investigators, R. Melvin, H. F. Dietz, G. S. Langford.

*Ansect Project*.—Supported by Interstate Chemical Company. Headquarters: Maryland Agricultural Experiment Station. Investigator, L. L. Williams.

*Organic Chemicals Investigations*.—Supported by Monsanto Chemical Company. Headquarters: Illinois Agricultural Experiment Station. Investigator, G. L. Hockenyos. Publication, Bulletin No. 31.

*Pyrethrum Sprays*.—Supported by J. C. Makepeace. Headquarters: Columbus, Ohio, and Wareham, Mass. Investigators, H. G. Walker, E. A. Richmond, and G. L. Walker.

*Nurserymen's Tape*.—Supported by Johnson & Johnson. Headquarters: University of Wisconsin. Investigator, S. S. Ivanoff.

*Kay Fungicides Project*.—Supported by Kay Laboratories, Inc. Headquarters: Delaware Agricultural Experiment Station. Investigators, D. S. Loveland, and J. S. Trumbower.

*Kerosene Household Sprays Investigations*.—Supported by Deep Rock Oil Corporation. Headquarters: Iowa State College. Investigator, H. H. Richardson. Publication, Bulletins Nos. 34 and 37.

*Cocoanut Oil Emulsion Studies*.—Supported by Research Corporation. Headquarters: Boyce Thompson Institute. Investigator, L. E. Dills.

*Volck Project*.—Supported by California Spray-Chemical Company. Headquarters: Kansas Agricultural Experiment Station, Manhattan, Kansas. Investigators, W. G. Bruce, H. L. Caler, and D. G. Hall. Publications, Bulletins Nos. 17, 22, and 35.

*Control of Fruit Moth by Oil Spray*.—Supported by California Spray-Chemical Corporation. Headquarters: Delaware Agricultural Experiment Station. Publication, Bulletin No. 41. Investigator D. McCreary.

*Codling Moth Studies, Washington*.—Supported by California Spray-Chemical Corporation. Headquarters: Washington Agricultural Experiment Station. Investigators, T. P. Strand, K. M. Gillies, and C. Miller.

*Codling Moth Studies, California*.—Supported by California Spray-Chemical Corporation. Headquarters: University of California, Berkeley. Investigator, Mr. Hensill.

*Oil Residues Project*.—Supported by California Spray-Chemical Corporation. Headquarters: New Hampshire Agricultural Experiment Station. Investigator, L. S. Groves.

*Diamalt Project*.—Supported by Standard Brands, Inc. Headquarters:

Maryland Agricultural Experiment Station. Investigator, W. A. Thorne. *Thiocide Project*.—Supported by Midwest Chemical Company. Headquarters: University of Illinois, Urbana. Investigators, G. L. Hockenyos and Mr. Blair.

*Organic Insecticides Investigations*.—Supported by Dow Chemical Company. Headquarters: Iowa State College. Investigator, J. F. Kagy.

*Sulphur Project*.—Supported by Mac Sulphur Company. Headquarters: Delaware Agricultural Experiment Station. Investigators, J. C. Carter, Raymond Russell.

*Iodine Project*.—Supported by Iodine Educational Bureau, Inc. Headquarters: New Jersey Agricultural Experiment Station. Investigator, C. H. Blasberg.

*Sharples Solvents Project*.—Supported by Sharples Solvents Corporation. Headquarters: Ohio Agricultural Experiment Station, Wooster, and Philadelphia. Investigator, M. M. Darley.

*Liquid Carbon Dioxide Project*.—Supported by Liquid Carbonic Corporation. Headquarters: Iowa State College and Chicago. Investigator, R. M. Jones. Publications, Bulletins Nos. 40 and 42.

*Copper Fungicides Project*.—Supported by California Spray-Chemical Corporation. Headquarters: New Jersey Agricultural Experiment Station. Investigator, A. C. Sessions.

*Organic Insecticides and Fungicides (Two projects)*.—Supported by National Aniline and Chemical Company, Inc. Headquarters: Delaware Agricultural Experiment Station, New Hampshire Agricultural Experiment Station, Florida, and Mississippi. Investigators, G. L. Walker and C. N. Priode.

*Halowax Project*.—Supported by Halowax Corporation. Headquarters: Ohio State University and Sanford, Florida. Investigator, E. P. Breaky. Publication, Bulletin No. 45.

*Copper Fungicides Project*.—Supported by Nichols Copper Company. Headquarters: Delaware Agricultural Experiment Station. Investigator, A. A. Nikitin.

*Copper in Plant Nutrition*.—Supported by Nichols Copper Company. Headquarters: Delaware Agricultural Experiment Station. Investigator, R. Russell. Publication, Bulletin No. 43.

*Repellents*.—Supported by Standard Oil Company of New Jersey. Headquarters: New Jersey Agricultural Experiment Station. Investigator, W. Moore.

*Sulphur Project*.—Supported by Freeport Sulphur Company. Headquarters: Delaware Agricultural Experiment Station. Investigator, P. D. Peterson. Publication, Bulletin No. 44.

*Sulphuric Acid in Weed Control Project*.—Supported by Freeport Sulphur Company. Headquarters: University Farm, Davis, California. Investigator, W. E. Ball.

*Sulphur and Carbon Bisulphide (Two projects)*.—Supported by Stauffer Chemical Company. Headquarters: Connecticut Agricultural Experiment Station and Massachusetts Agricultural Experiment Station. Investigators, M. V. Anthony and T. T. Ayers.

*Plant Extracts as Insecticides*.—Supported by S. B. Penick & Company. Headquarters: New Hampshire Agricultural Station. Investigator, O. J. Smith.

*Copper Fungicides Project*.—Supported by Shepherd Chemical Company. Headquarters: New Hampshire Agricultural Experiment Station and Delaware Agricultural Experiment Station.

W. C. O'KANE,  
*Chairman, Board of Governors*



# PHYTOPATHOLOGY

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## CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

### I. SOME EFFECTS OF TRYPSIN

W. M. STANLEY

(Accepted for publication July 26, 1934)<sup>1</sup>

#### INTRODUCTION

There has been little work reported on the action of enzymes on animal and plant viruses, despite the fact that digestion of a virus with a proteolytic enzyme would serve as an indication that viruses are proteins. Vinson and Petre (15) and Lojkin and Vinson (5) reported that emulsin, pepsin, and yeast extract had no effect on the infectivity of tobacco-mosaic virus, but that trypsin and papain inactivated this virus and that the inactivation caused by trypsin was probably due to the hydrolytic action of the enzyme. Somewhat later Matsumoto and Somazawa (6) reported that the infective principle and certain precipitating agents of juice from tobacco plants infected with mosaic withstood the action of rather large amounts of trypsin fairly well, although certain antigenic properties were greatly impaired. Caldwell (1) found that trypsin, when added to aucuba-mosaic virus, caused a loss in infectivity, but that some of the infectivity of the virus returned when the mixture was heated, a result with which Vinson (14) has recently agreed.

If the virus were actually split or digested by trypsin, as first suggested by Vinson, reactivation by heat would be improbable. Furthermore, if the inactivation were due to proteolysis one would expect pepsin, rather than trypsin, to be an active proteolytic agent. Trypsin usually fails to digest native proteins, whereas pepsin is usually able to digest both native and denatured proteins. It was decided, therefore, to reinvestigate the action of trypsin and of pepsin on the virus of tobacco mosaic. The present paper records the results of a study of some effects of trypsin upon this virus.

#### MATERIALS AND METHODS

Two preparations of tobacco-mosaic virus were used in the experiments to be reported. One consisted of untreated juice from *Nicotiana tabacum*

<sup>1</sup> Published at the expense of The Rockefeller Institute for Medical Research out of the order determined by date of receipt of the manuscript.

L. var. Turkish infected with tobacco mosaic. The other, which was used in most of the experiments, was prepared from infectious juice by a modification of the method of Vinson and Petre (16). Juice from Turkish tobacco plants infected with mosaic was precipitated with lead acetate and eluted with 0.1 M potassium phosphate at pH 7. The volume of this purified preparation was so adjusted that its infectivity was comparable to that of the untreated infectious juice. Dilutions of purified virus were made with 0.1 M phosphate at pH 7 and dilutions of untreated infectious juice were made with distilled water.

The trypsin used was a highly purified crystalline preparation<sup>2</sup> and commercial Fairchild trypsin. It is believed that the crystalline trypsin consists of only one enzyme, whereas commercial trypsin is a mixture of several enzymes (8). Because of this the crystalline trypsin preparation was used in the greater part of the work. It was employed in the form of a solution in 0.01 N hydrochloric acid, usually containing 0.1 mg. trypsin nitrogen per cc. The addition of this solution to the virus preparations caused little or no change in the hydrogen-ion concentration of the latter, since the preparations were well buffered. The powdered commercial trypsin was added directly to the virus preparations. One drop of toluene per 10 cc. of solution was added as a preservative. Controls for the commercial trypsin consisted of virus preparations that had been treated in exactly the same manner as the trypsin-virus solutions, except that no trypsin was added. Controls in the case of the crystalline trypsin consisted of virus preparations that had been treated in exactly the same manner as the trypsin-virus solutions, except that 0.01 N HCl was added instead of the trypsin solution. With the above exceptions, the protein-treated solutions and the corresponding control solutions were prepared, incubated, and inoculated in the same manner and under identical conditions.

The concentration of the virus was estimated by the local-lesion method developed by Holmes (2) and by Price (9) for *Nicotiana glutinosa* L. and *Phaseolus vulgaris* L. variety Early Golden Cluster, respectively. Because of the desirability of measuring as accurately as possible the relative infectivity of the virus in the protein-treated solution and in the control, the half-leaf method (2, 11) of inoculation was used whenever possible. The average number of lesions produced when the preparation of virus and protein was inoculated on one-half of the leaf was compared with the average produced by the control virus preparation on the other half of the same leaf. The diminution of the infectivity of a given virus sample by means of extraneous materials or by means of a change in hydrogen-ion

<sup>2</sup> The writer is indebted to Dr. J. H. Northrop, Dr. M. Kunitz and Dr. M. L. Anson for the purified proteins used in this investigation.



concentration, as well as the opposite effect, consisting of the enhancement of the infectivity of a given virus preparation by similar means, need always be taken into account whenever the relative amount of virus in a sample is under consideration. It was found, for example, that at some hydrogen-ion concentrations, and in the presence of certain materials, some susceptible plants failed to produce lesions when inoculated with virus preparations, even though virus was present in an active condition and in high concentration, as was demonstrated by inoculation of other species of test plants. Therefore, virus preparations which gave negative results on one plant species were tested at a favorable hydrogen-ion concentration on a number of different species of plants before the absence of virus infectivity was accepted. Even then it is possible, of course, that virus may be present, but that it fails to infect the plants.

In most of the experiments the same virus preparation and its control were tested on 6 or more plants of *Phaseolus vulgaris* having 12 or more primary leaves and on 1 or 2 plants of *Nicotiana glutinosa* having 5 leaves each. Inoculation was accomplished by rubbing the upper surface of one-half of each leaf with a small piece of folded bandage gauze moistened with the test solution. The other half of the same leaf was similarly treated with the control solution and the leaf was then washed in running tap water. The leaf was held under the stream of water so that the wash water ran from the control half towards the protein-treated half and not in the reverse direction. This was done to prevent trypsin in the wash water from coming in contact with the control half of the leaf. In certain instances the tests were made both by the half-leaf and whole-leaf methods to detect possible errors from this source. The lesions, which usually appeared within 48 hours after inoculation, were counted at the end of 4 or 5 days. Unless otherwise specified, all inoculations in the following experiments were made by this rubbing method.

#### EXPERIMENTAL

##### Studies on the Proteolytic Action of Trypsin

*Comparison of the Effects of Trypsin and Trypsinogen.* Table 1 gives the results of a series of experiments designed to demonstrate, under various conditions, the effect of crystalline trypsin and crystalline trypsinogen on the infectivity of a purified preparation of tobacco-mosaic virus. Trypsinogen, which is trypsin before it has been activated, has no proteolytic activity. In this experiment, in which plants of *Nicotiana tabacum* were also used as test plants, the inoculation of these plants was made by puncturing 1 leaf of each plant with 5 pins that had been dipped in the test solution. The inoculation of *N. glutinosa* and *Phaseolus vulgaris* was

TABLE 1.—*Comparison of the effects of trypsin and trypsinogen on the infectivity of a purified preparation of tobacco-mosaic virus.*

Dilution →	Tested ↓	Undiluted			1-10			1-100			1-1000
		P. vulgaris	N. glutinosa	Turkish % takes	P. vulgaris	N. glutinosa	P. vulgaris	P. vulgaris	N. glutinosa	P. vulgaris	
* Virus pH 7.0	L <sub>1</sub>	142.0 <sup>a</sup>	100.0 <sup>b</sup>	39.5 <sup>c</sup>	77.5	36.2	49.0	3.26	5.20		
	R <sub>1</sub>	84.0	101.0		59.4	24.5	42.6	23.1	1.94		
	Av.	113.0	100.5		53.6	31.8	45.8	13.2	3.5		
	L <sub>1</sub>	51.2	81.6	10.0	13.7	40.1	3.72	6.4	2.56		
Heated virus 70° C.	R <sub>1</sub>	32.2	84.0		10.6	69.0	5.30	3.6	2.75		
	Av.	41.7	82.8		6.7	54.5	4.5	5.0	2.6		
	L <sub>1</sub>	7.9	18.1	6.0	16.1	4.4	0.16				
	R <sub>1</sub>	28.7	26.4		19.6	22.6	0.00				
Trypsin- virus	Av.	18.3	22.2		2.2	8.7	0.08				
	L <sub>10</sub>	14.7	29.3		7.05	13.3	1.94				
	R <sub>11</sub>	9.05	14.4		12.7	15.6	4.75				
	Av.	11.9	21.8		10.3	14.4	3.3				
Heated trypsin- virus 70° C.	L <sub>2</sub>	0.00	1.40	0.00	0.15	1.00	1.24	3.8	0.05		
	R <sub>3</sub>	0.00	2.74		0.12	4.13	0.50	1.4	0.33		
	Av.	0.00	2.07		0.1	2.0	0.9	2.6	0.2		
	L <sub>3</sub>	0.00	4.4	1.0	0.00	13.4	0.41	0.13	0.31		
Heated trypsin- virus 70° C.	R <sub>6</sub>	0.20	10.8		0.06	11.3	0.00	0.93	0.11		
	Av.	0.10	7.6		0.06	12.3	0.2	0.5	0.2		
	L <sub>3</sub>	0.00	15.7		0.35	3.30	0.00				
	R <sub>6</sub>	0.05	12.3		0.22	2.20	0.00				
Heated trypsin- virus 70° C.	Av.	0.02	14.0		0.06	1.90	0.00				
	L <sub>11</sub>	3.40	3.54	3.0	0.2	2.5	0.00				
	R <sub>11</sub>	2.75	6.46		2.20	4.87	0.53				
	Av.	3.10	5.0		1.94	6.74	1.06				
					2.90	5.8	0.8				

TABLE 1.—(Continued)

Dilution →	Tested	Undiluted				1-10		1-100		1-1000
		P. vulgaris	N. glutinosa	Turkish % takes	P. vulgaris	N. glutinosa	P. vulgaris	N. glutinosa	P. vulgaris	P. vulgaris
Tested on →	↓									
	Atf once	L <sub>9</sub> R <sub>9</sub>	0.13 0.13	0.0	2.44 1.12 0.22 1.2	1.00 1.33 0.50 0.9	0.78 0.15 0.5	0.80 1.07 0.9	0.50 0.00 0.25	
	Av.	3.8	0.13							
Trypsin- ogen-virus	After† 6 days	L <sub>9</sub> R <sub>9</sub>	0.47 0.26	0.0	0.00 0.44 0.22 0.2	0.33 1.47 0.9	0.06 0.16 0.1	0.53 0.80 0.7	0.05 0.12 0.13	
	Av.	0.20	0.36							
	Atf once	L <sub>9</sub> R <sub>9</sub>	0.19 2.40		0.16 0.58 0.28 0.3	0.10 0.00 0.10 0.07	0.05 0.11 0.08			
	Av.	1.30	0.00							
Heated trypsin- ogen-virus 70° C.	After† 6 days	L <sub>12</sub> R <sub>10</sub>	0.66 0.80	0.0	0.06 0.00 0.16 0.07	0.80 0.47 0.6	0.11 0.06 0.08			
	Av.	0.10	0.73							

<sup>a</sup> Numbers represent the average number of lesions on 12 or more half-leaves of *P. vulgaris* var. Early Golden Cluster.

<sup>b</sup> Numbers represent the average number of lesions on 5 or more half-leaves of *N. glutinosa*.

<sup>c</sup> Numbers represent the per cent of infected Turkish tobacco plants out of 50 or 100 inoculated plants.

<sup>d</sup> Tryptic activity unchanged.

<sup>e</sup> Decreased tryptic activity.

<sup>f</sup> No tryptic activity.

by the rubbing method previously described. Since it was desired to find the relation between the infectivity of the untreated virus and the 2 treated virus solutions, as well as the relation between the 2 treated virus solutions, each solution was tested against each of the other 2 solutions on leaf-halves. This was repeated after heating and was also repeated at the end of 6 days, both before and after heating. The designations in column 3 refer to the leaf-half on which the various solutions were tested and the average of such tests. For example, the first horizontal row of numbers designated by  $L_1$  represents the average number of lesions per leaf produced on the left sides of the leaves by various concentrations of the virus, and the row of numbers designated by  $R_1$  represents the average number of lesions produced on the right sides of these same leaves by the trypsinogen-virus solution. Likewise,  $R_2$  represents the average number of lesions caused by the same virus preparation on the right sides of the leaves, and  $L_2$ , the average number of lesions caused by the trypsin-virus solution on the left sides of the same leaves. Each solution was tested twice, once on the right halves of one set of leaves and once on the left halves of another set of leaves. A third test was included with all solutions at a 1-10 dilution. The horizontal rows, marked "av.," represent the average of all tests of a given solution at a given dilution and are included in order that a rapid comparison of any two solutions may be made.

The results given in table 1 show that the reduction in infectivity of the virus by both trypsin and trypsinogen took place immediately. The infectivity of the virus-trypsin mixture increased on incubation for 6 days at 37° C., or on heating for 20 minutes at 70° C. It also increased on dilution, more lesions being produced when the virus-trypsin solution was diluted 1-100 than when used undiluted. The increase in infectivity by heating the virus-trypsin solution is similar to the results obtained by Caldwell (1) with aucuba-mosaic virus.

Since the digestion of substances by enzymes is a time reaction, it would appear that the loss of infectivity of virus is not due to the proteolytic activity of trypsin, inasmuch as it took place immediately upon the addition of the trypsin. The fact that the infectivity of the virus-trypsin solution increased on standing, on dilution, and on heating is further evidence that the loss of infectivity is not due to the proteolytic activity of the trypsin. The loss of virus infectivity on addition of trypsinogen is not due to proteolysis, since trypsinogen possesses no proteolytic activity.

The tryptic activity of the solution of trypsin was determined by means of the formol titration, both before and after addition to the virus, and no change was found. Tryptic activity decreased in the trypsin-virus solution after heating and was completely lost after standing for 7 weeks. No tryptic activity was found in any of the trypsinogen solutions at any time.

Since trypsinogen caused a loss of infectivity of the virus, it became of interest to determine whether other proteins would have the same effect. Table 2 gives the results of experiments with 5 different proteins. Here again the protein-treated virus was tested on one side of a leaf against an untreated control on the other side. The untreated virus preparation used in these tests gave an average of about 75 lesions per half-leaf. The results show that egg albumen, pepsin, and gelatin had only a slight effect on the infectivity of the virus at pH 7, whereas globin and commercial

TABLE 2.—*Effect of different proteins on the infectivity of tobacco-mosaic virus*

	pH of solution	Tested on	Immediately	After 3 days at 37° C.	After 7 days at 37° C.	After 14 days at 37° C.	After 25 days at 37° C.
Virus + egg albumin (3 mg. per cc.)	7.0	Phaseolus vulgaris	90.5 <sup>a</sup>				
		Nicotiana glutinosa	16.1				
Virus + globin (16 mg. per cc.)	5.8	Phaseolus vulgaris	0.8				
Virus + Parke-Davis pepsin (2 mg. per cc.)	7.0	Nicotiana glutinosa	108.0	84.7	75.1	126.0	
		Nicotiana glutinosa	89.0	93.9	69.0	117.0	
Virus + gelatin (2 mg. per cc.)	7.0	Phaseolus vulgaris	83.5	80.3	90.0	95.0	
		Nicotiana glutinosa	30.9	66.0	41.4		104.5
Virus + Fairchild trypsin (2 mg. per cc.)	7.0	Phaseolus vulgaris	0.0	3.4	7.6		7.5 <sup>b</sup>

<sup>a</sup> The numbers represent the average number of lesions on halves of 12 or more leaves of *P. vulgaris* var. Early Golden Cluster and of 5 or more leaves of *N. glutinosa*, expressed as a percentage of the number of lesions obtained with the appropriate control.

<sup>b</sup> This solution was found to possess no tryptic activity.

trypsin caused a great loss in infectivity. Globin, like trypsin and trypsinogen, is peculiar in that it is one of the few proteins that have isoelectric points above pH 7. It may be significant that these three proteins cause a marked decrease in virus infectivity, whereas the proteins that have isoelectric points considerably below pH 7 have no such effect.

In this experiment it may be noted that the commercial trypsin-virus solution possessed no tryptic activity at the end of 25 days at 37° C., whereas the infectivity of the virus as tested on *Nicotiana glutinosa* had been regained until it was comparable to that of the untreated control solu-

TABLE 3.—*Recovery of virus infectivity in a trypsin-virus solution by means of pepsin*

	pH 3		pH 7	
	<i>N. glutinosa</i>	<i>P. vulgaris</i>	<i>N. glutinosa</i>	<i>P. vulgaris</i>
Virus	65.6 <sup>a</sup>	3.1 ←	74.2	87.2
Virus + trypsin (0.1 mg. trypsin N per cc.)	10.1	0.0 ←	11.6	0.0
Virus + trypsin + pepsin (0.01 mg. pepsin N per cc.)	↓ 5.0	0.0		
Virus + trypsin + pepsin (After 2 days at 37° C.) (No tryptic activity)	↓ 46.5		57.5	13.9

<sup>a</sup> The numbers represent the average number of lesions on 12 or more leaves of *P. vulgaris* var. Early Golden Cluster or 10 or more leaves of *N. glutinosa*.

tion. The disappearance of the trypsin and the reactivation of the virus is probably due to the slow denaturation of trypsin which occurs at pH 7 and the digestion of the denatured trypsin by the remaining active trypsin. This process may be assumed to continue until almost all the trypsin has been digested, and as the trypsin is thus removed, the virus is able to manifest its infectivity.

*Recovery of Infectivity by Peptic Digestion.* Since from the experiments reported above, it appears that the gradual removal of trypsin causes a recovery of virus infectivity, it was decided to determine whether the infectivity could be regained by the rapid digestion of trypsin with pepsin. Table 3 gives the results of the treatment of a trypsin-virus solution with pepsin. In this experiment a purified virus solution was tested at pH 3 and 7, giving the results shown in the first row of figures. To the solution at pH 7 was then added enough of a solution of crystalline trypsin to make 0.1 mg. trypsin nitrogen per cc. This mixture was tested on half-leaves of *Phaseolus vulgaris* and *Nicotiana glutinosa* against the proper controls at pH 3 and 7. It may be seen from table 3 that the preparation produced no lesions on *P. vulgaris*, yet gave an average of 11.6 lesions on *N. glutinosa*. The significance of this differentiation, which has been encountered throughout the work, will be considered in detail in a subsequent section. Enough crystalline pepsin to make 0.01 mg. pepsin nitrogen per cc. was added to the virus solution that had been brought to pH 3. The solution was tested for infectivity on *N. glutinosa* and *P. vulgaris* at pH 3 immediately after the addition of the pepsin. It was then incubated for 2 days at 37° C. and tested at pH 3 and 7 for infectivity and also for tryptic activity. No tryptic activity was found, but the infectivity of the virus had been re-

gained to such an extent that the solution now produced, at pH 7, an average of 13.9 lesions on *P. vulgaris* and 57.5 lesions on *N. glutinosa* as compared to an average of 0.0 and 11.6 lesions, respectively, before the treatment with pepsin. This gain in the infectivity of the virus in the trypsin and pepsin treated solution was such that the solution produced about 16 per cent as many lesions on *P. vulgaris* and 77 per cent as many lesions on *N. glutinosa* as did the original untreated virus solution.

There remained the possibility, however, that the pepsin or the split products of trypsin might have a stimulating effect on the virus, which would account for the increase in infectivity as measured on the 2 test plants. In order to eliminate this possibility pepsin was allowed to act upon trypsin for 66 hours at pH 3 in the relative concentrations mentioned above. Then 1 cc. of this pepsin-trypsin digest was added to 4 cc. of the virus solution and this mixture was tested immediately on leaf-halves against a control, using *Phaseolus vulgaris* and *Nicotiana glutinosa*. Instead of causing an increase in infectivity, this digest caused a slight reduction in the average number of lesions on *N. glutinosa* and a somewhat greater reduction in the number of lesions on *P. vulgaris*. It appears, therefore, that the gain in virus infectivity, which was measured on these plants and was caused by pepsin, represents the minimum rather than the maximum gain in infectivity. However, *N. glutinosa* was least affected by the digest, so the measurements on this plant can be regarded as being more nearly indicative of the real gain in infectivity. Since the virus in the trypsin-pepsin digest gave an average of about 77 per cent as many lesions as did the virus in the untreated control solution, it is obvious that much of the virus infectivity was regained by digestion of the trypsin with pepsin.

*The Effect of Different Hydrogen-Ion Concentrations.* If the loss of virus infectivity on addition of trypsin is due to proteolysis, it should take place only at hydrogen-ion concentrations at which trypsin is active. Trypsin has its maximum proteolytic activity between pH 7.5 and 9.5 and is inactive below about pH 6. Therefore, the effect of trypsin on the infectivity of virus was determined at pH 2.1, 2.7, 4.0 and 5.0, hydrogen-ion concentrations at which trypsin is inactive and also at pH 6.7, 8.0, 10.2 and 10.8, hydrogen-ion concentrations at which trypsin is active. In this experiment a sample of virus, a solution of 0.1 M phosphate and a solution of 0.1 M phosphate containing 0.5 mg. of trypsin nitrogen per cc. were prepared at each of the above hydrogen-ion concentrations. Each virus sample was divided into two equal portions and at each hydrogen-ion concentration an equal volume of 0.1 M phosphate was added to one portion and an equal volume of 0.1 M phosphate containing 0.5 mg. of trypsin nitrogen per cc. was added to the other portion. The hydrogen-ion concentration of each mixture was the same, therefore, as that of the virus sample used in its preparation. The virus preparations at the 8 different hydrogen-

ion concentrations were tested by the half-leaf method against the 8 virus preparations containing 0.25 mg. of trypsin nitrogen per cc. The results (Table 4) show that the hydrogen-ion concentration of the virus preparation has little or no effect on the loss of infectivity, since it took place at all 8

TABLE 4.—Effect of hydrogen-ion concentration on the loss of virus infectivity upon addition of trypsin

Tested at pH →		2.1	2.7	4.0	5.0	6.7	8.0	10.2	10.8
<i>P. vulgaris</i>	Virus	7.6*	3.3	10.2	33.4	39.6	48.2	57.8	35.3
	Virus plus trypsin (0.25 mg. N per cc.)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>N. glutinosa</i>	Virus	19.4	40.1	48.2	47.4	61.6	41.1	9.2	6.0
	Virus plus trypsin (0.25 mg. N per cc.)	1.2	3.3	3.8	3.2	2.0	1.8	1.0	0.0

\* The numbers represent the average number of lesions on 12 half-leaves of *P. vulgaris* var. Early Golden Cluster or 10 half-leaves of *N. glutinosa*.

hydrogen-ion concentrations. This suggests that the loss of infectivity is not due to the proteolytic activity of trypsin, since it is inactive proteolytically at pH 2.1, 2.7, 4.0 and 5.0.

#### Studies on Other Effects of Trypsin

If the loss in infectivity of virus on addition of trypsin is not due to proteolysis, it must be explained on some other basis. It seems necessary to assume that trypsin affects either the virus, or the plant, or both. Experiments were therefore made to determine whether trypsin has a direct action on the test plant. Other experiments were made to determine the effect of trypsin on the infectivity of several different viruses, and on the infectivity of tobacco mosaic virus when used to inoculate different species of plants.

*The Effect of Different Amounts of Trypsin on the Susceptibility of Plants to the Virus of Tobacco Mosaic.* If trypsin acts directly on the plant cells it might be possible to study its action by varying the amount that is added to the virus. Table 5 shows the results of experiments in which solutions of crystalline trypsin were added to untreated juice from mosaic-diseased tobacco plants at pH 5.6 and to a purified virus solution at pH 6.8, and tested immediately with controls on *Phaseolus vulgaris* and *Nicotiana glutinosa*. It may be seen that, while 0.005 or more milligrams



TABLE 5.—*Effect of varying amounts of trypsin on the infectivity of tobacco mosaic virus*

Milligrams of trypsin N per cc. —→		0	0.005	0.05	0.25	0.5
Untreated juice from mosaic tobacco plants. Diluted 1 to 6.	<i>P. vulgaris</i>	230.0 <sup>a</sup>	17.4	1.0	0.0	0.0
	<i>N. glutinosa</i>	303.0	168.0	78.4	21.6	11.8
Purified virus (pH 5.6)	<i>P. vulgaris</i>	121.0	3.4	0.8	0.0	0.0
	<i>N. glutinosa</i>	194.0	88.5	2.0	2.2	0.0

<sup>a</sup> Numbers represent the average number of lesions on 16 leaves of *P. vulgaris* var. Early Golden Cluster or 10 leaves of *N. glutinosa*.

of trypsin nitrogen per cc. has a decided effect when either solution is used, it is more marked in the case of the purified virus solution. Additional tests have demonstrated that even 0.0005 mg. of trypsin nitrogen is very effective when added to a purified virus solution. Lojkin and Vinson (5) reported that they were unable to obtain inactivation of the virus by means of trypsin when they used untreated juice from mosaic-diseased tobacco plants. This difference in results may be due to the use of different enzyme preparations.

TABLE 6.—*Effect of varying amounts of trypsin and virus on infectivity in Phaseolus vulgaris and Nicotiana glutinosa*

Final dilution of virus —→		1-2	1-20	1-200
Virus plus 0.25 mg. trypsin N per cc.	<i>P. vulgaris</i>	0.0 <sup>a</sup>	0.0	0.0
	<i>N. glutinosa</i>	10.8	1.9	0.1
Virus plus 0.125 mg. trypsin N per cc.	<i>P. vulgaris</i>	0.0	0.0	0.0
	<i>N. glutinosa</i>	14.3	5.5	0.3
Virus plus 0.025 mg. trypsin N per cc.	<i>P. vulgaris</i>	0.5	0.5	0.0
	<i>N. glutinosa</i>	46.6	29.6	1.4
Virus plus 0.0025 mg. trypsin N per cc.	<i>P. vulgaris</i>	15.8	6.9	0.5
	<i>N. glutinosa</i>	45.8	55.5	6.2
Virus plus 0.00125 mg trypsin N per cc.	<i>P. vulgaris</i>		17.8	14.7
	<i>N. glutinosa</i>		46.8	8.7
Virus plus no trypsin	<i>P. vulgaris</i>	55.0	49.0	14.9
	<i>N. glutinosa</i>	65.0	65.0	8.2

<sup>a</sup> The numbers represent the average number of lesions on 12 or more half-leaves of *P. vulgaris* var. Early Golden Cluster or 5 or more half-leaves of *N. glutinosa*.

Studies were then made with varying amounts of virus as well as of trypsin. To purified solutions of virus in 0.1 M phosphate buffer at pH 6.8 were added equal volumes of 0.01 M HCl containing from zero to 0.5 mg. of trypsin nitrogen per cc. The virus solution was sufficiently well buffered that the addition of the acid caused no appreciable change in the pH. The final dilution of the virus solution was 1 part in 2, 1 in 20, and 1 in 200. The amount of trypsin was varied from 0.25 to 0.0025 mg. trypsin nitrogen per cc. It may be seen from table 6 that trypsin caused a decrease in the number of lesions at all concentrations used and that this decrease was greater with *Phaseolus vulgaris* than it was with *Nicotiana glutinosa*. The two highest concentrations of trypsin caused the virus solution to lose completely its infectivity for *P. vulgaris*, although these same solutions produced lesions on *N. glutinosa*. This phenomenon, as was previously mentioned, has been encountered throughout the work. The fact that it is possible to prepare a virus-trypsin solution that will produce local necrotic lesions on one plant species and yet fail to produce lesions on a second equally or more susceptible species is a definite indication that trypsin is affecting the second test plant. It has been demonstrated repeatedly that a virus solution that has been diluted with distilled water or phosphate at pH 7 so that it will give an average of approximately 10 lesions per leaf on *N. glutinosa* will also give an average of at least 10 and usually more lesions per leaf on *P. vulgaris*. The results of the tests of the control virus solutions as given in tables 1, 6, and 7 may be cited as examples. It may be seen from these tables that, at this concentration of virus, plants of *P. vulgaris* respond to inoculation with as many lesions as do *N. glutinosa* plants. Therefore, the production of lesions on *N. glutinosa* on inoculation with the virus-trypsin solution and the failure of the same solution to produce lesions on *P. vulgaris* is definite evidence that trypsin is affecting the plants of *P. vulgaris*.

Similar results were obtained when a more concentrated virus solution, which permitted greater dilution, was used, as may be seen from table 7. In this experiment the trypsin nitrogen concentration was varied between 0.0005 mg. and 0.1 mg. per cc. and the dilutions of virus were carried out to 1 part in 200,000. In many instances at higher trypsin concentrations or lower virus concentrations, solutions were obtained that gave lesions on *Nicotiana glutinosa* and yet failed to produce lesions on *Phaseolus vulgaris*. In order to gain additional information concerning the difference in response to trypsin of these two plant species an attempt to correlate the data given in table 7 was made. The number of lesions obtained with the control or untreated virus solution was regarded as 100 per cent at each dilution and the percentage drop in the number of lesions from this number at each dilution was calculated. This percentage drop in the number of

TABLE 7.—*The effect of a constant amount of trypsin on varying amounts of virus*

Dilution of virus →	Test plant ↓	1-2	1-20	1- 200	1- 2000	1- 20,000	1- 200,000
Virus	<i>P. vulgaris</i>	295.8 <sup>a</sup>	135.9	65.6	11.4	1.16	0.3
	<i>N. glutinosa</i>	214.0	97.0	32.2	2.05	0.2	0.0
Virus plus 0.0005 mg. trypsin N per cc.	<i>P. vulgaris</i>	95.5	30.5	6.5	0.28	0.0	0.0
Virus plus 0.001 mg. trypsin N per cc.	<i>P. vulgaris</i>	54.0	18.4	2.0	0.0	0.0	0.0
	<i>N. glutinosa</i>	147.0	60.2	28.7	1.6	0.2	0.0
Virus plus 0.002 mg. trypsin N per cc.	<i>N. glutinosa</i>	141.5	67.3	10.6	1.35	0.06	0.0
Virus plus 0.005 mg. trypsin N per cc.	<i>P. vulgaris</i>	15.4	3.6	0.4	0.0	0.0	0.0
	<i>N. glutinosa</i>	176.0	42.3	9.2	1.3	0.1	0.0
Virus plus 0.05 mg. trypsin N per cc.	<i>P. vulgaris</i>	0.5	0.0	0.0	0.0		
	<i>N. glutinosa</i>	47.3	6.6	2.8	0.4	0.09	0.0
Virus plus 0.1 mg. trypsin N per cc.	<i>P. vulgaris</i>	0.5	0.0	0.0			
	<i>N. glutinosa</i>	16.5	2.7	1.3	0.1		

<sup>a</sup> The numbers represent the average number of lesions on 12 or more half-leaves of *P. vulgaris* var. Early Golden Cluster or *N. glutinosa*.

lesions was plotted (see Figs. 1 and 2) against the dilution of the virus on a double logarithmic scale, for each trypsin concentration and for each test plant. It may be seen from figure 1 that with this virus solution, concentrations of from 0.001 mg. to 0.1 mg. trypsin nitrogen per cc., gave lines approximately paralleling that of the control when tested on *N. glutinosa*. This indicates that these trypsin concentrations produce constant percentage reductions in the number of lesions on *N. glutinosa*. The results as given in figure 2 show that trypsin concentrations of 0.0005 mg. trypsin nitrogen per cc. or more gave progressively increasing percentage drops in the number of lesions when tested on *P. vulgaris*. The difference in the response of the two test plants to trypsin may be seen by a comparison of the curves for trypsin nitrogen concentrations of 0.001 and 0.005 mg. in figures 1 and 2. In these cases a constant reduction at all dilutions was obtained on *N. glutinosa*, whereas the same solutions gave progressively increasing reductions on dilution of the virus when tested on *P. vulgaris*.

The fact that it is possible to obtain a fairly constant reduction in the number of lesions on *Nicotiana glutinosa* at several dilutions of virus with a given constant amount of trypsin seems to indicate that trypsin is acting

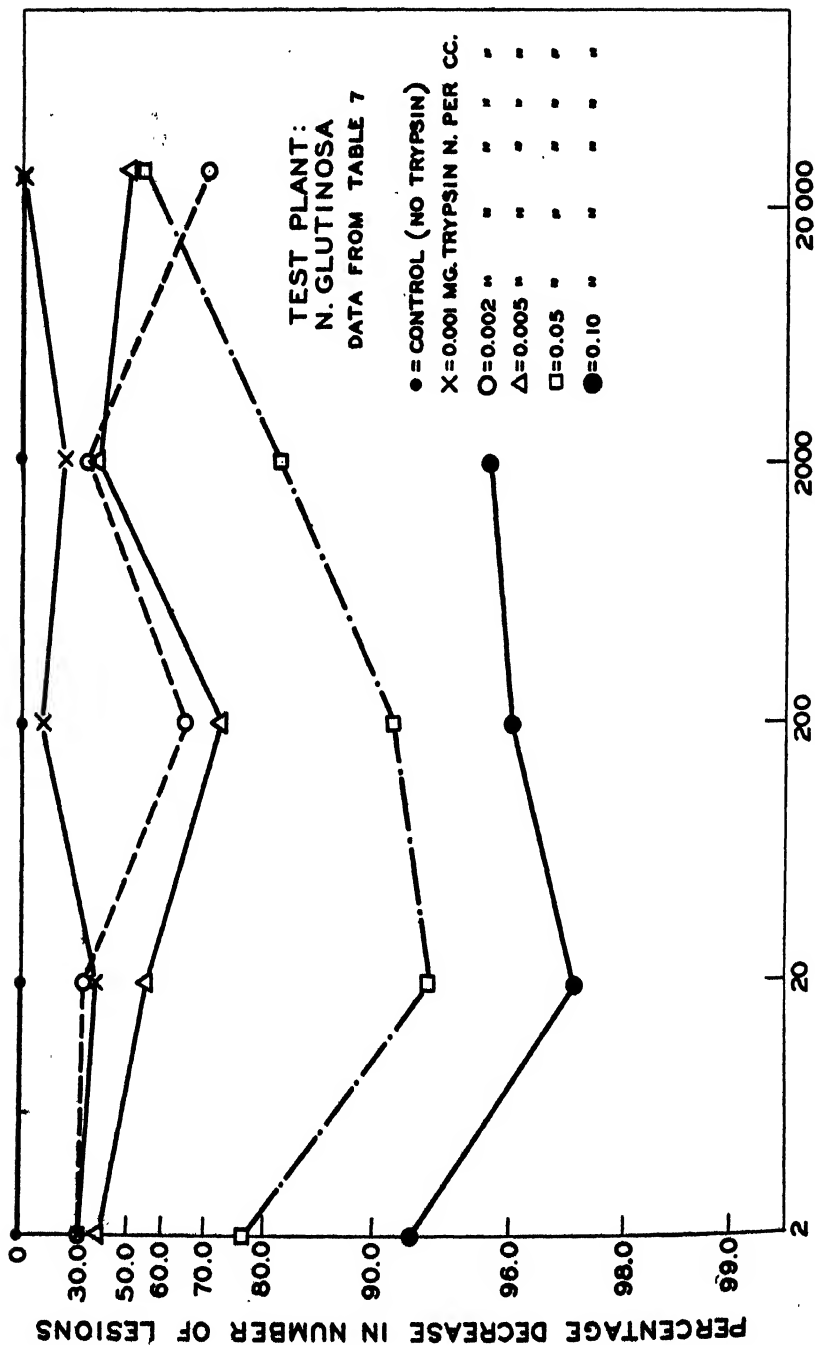


FIG. 1. Graph showing the percentage decrease in the average number of lesions on 12 or more leaves of *Nicotiana glutinosa* L. upon inoculation with tobacco-mosaic virus at several dilutions in the presence of 5 different concentrations of trypsin. In general the lines are parallel. This shows that at these concentrations, trypsin causes a fairly constant percentage reduction in the number of lesions on *N. glutinosa* upon dilution of the virus.

on the plant. However, the fact that at certain higher concentrations of trypsin it is possible to demonstrate that a given amount of trypsin causes a progressively increasing percentage drop in the number of lesions, on dilution of the virus, indicates that here there may be some action of trypsin on the virus, since as the virus concentration is reduced, the inhibitory effect of the trypsin becomes greater. However, if the progressively in-

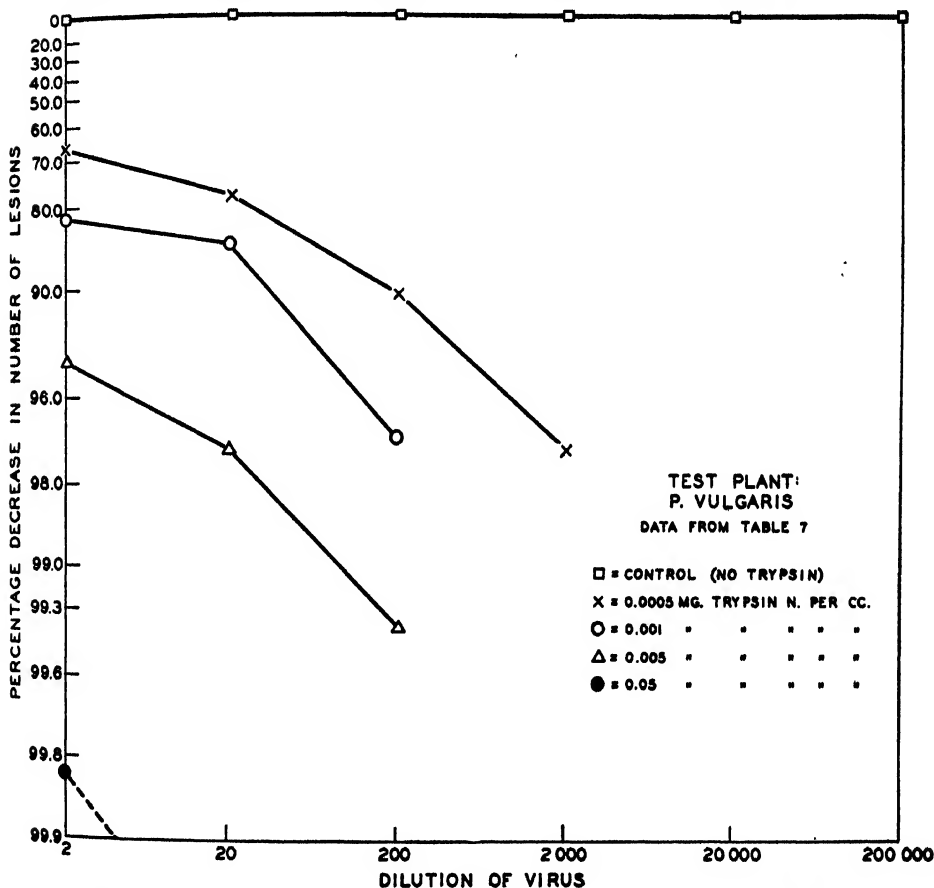


FIG. 2. Graph showing the percentage decrease in the average number of lesions on 12 or more leaves of *Phaseolus vulgaris* L. upon inoculation with tobacco-mosaic virus at several dilutions in the presence of 4 different concentrations of trypsin. The drop in the 4 lines depicting 4 trypsin concentrations shows that at these concentrations, trypsin causes progressively increasing percentage reductions in the number of lesions on *P. vulgaris* upon dilution of the virus.

creasing drop in the number of lesions obtained when a virus solution containing 0.001 mg. trypsin nitrogen is tested on *Phaseolus vulgaris* is to be regarded as evidence that trypsin is acting on the virus, it is difficult to

understand why this same drop, indicative of effect on virus, does not appear when this same solution is tested on *N. glutinosa*. Here the evidence seems to indicate that trypsin is affecting only the test plant, since the percentage reduction in the number of lesions is the same at several dilutions of the virus. Some secondary and as yet undemonstrated factor may be causing this phenomenon. There seems to be no doubt, however, that trypsin does act directly on *N. glutinosa* and *P. vulgaris* to reduce their susceptibility to the virus of tobacco mosaic, and that the intensity of the action of trypsin is not the same for these two plant species.

*Effect of Trypsin on the Susceptibility of Different Species of Plants to the Virus of Tobacco Mosaic.* Since trypsin was found to affect the susceptibility of plants to the virus of tobacco mosaic, the action being more pronounced with *Phaseolus vulgaris* than with *Nicotiana glutinosa*, it seemed possible that the action might vary to an even greater extent with other plants. In fact, it did not appear impossible that some plant might be entirely resistant to trypsin and hence be as susceptible to virus plus trypsin as to virus alone. In order to test this possibility the right halves of leaves of one or more plants of *Nicotiana acuminata* Hook., *N. langsdorffii* Schrank, *N. rustica* L. var. Winnebago, *N. tabacum* var. *purpurea*  $\times$  *glutinosa*, *Datura stramonium* L., *Solanum melongena* L. var. Black Beauty, *S. pseudo-capsicum* L., *Physalis angulata* L., and *Capsicum frutescens* L. were rubbed with a sample of purified virus. The left halves of the same leaves were rubbed with a similar sample of virus plus 0.5 mg. of trypsin nitrogen per cc. The test was repeated, using a sample of untreated infectious juice from mosaic-diseased Turkish tobacco plants instead of the purified virus. This sample was tested against untreated infectious juice plus 0.5 mg. trypsin nitrogen per cc. The results, some of which may be judged from figure 3, were the same in all instances. The virus caused many necrotic lesions on the right sides of the leaves where inoculation was with virus alone, and none or at most 1 or 2 lesions on the left sides where inoculation was carried out in the presence of trypsin. The plants listed above represent a rather wide range of species, yet all of the plants were found to be affected by trypsin. Therefore, although the intensity of the inhibitory action of trypsin on the susceptibility of plants to the virus of tobacco-mosaic may vary somewhat with the species of test plant used, the inhibitory action appears to be more or less general, since all plants used were affected by trypsin.

*Effect of Adding Trypsin after Inoculation with Virus.* It seemed of interest to determine whether a solution of trypsin, rubbed or sprayed on the leaves of inoculated plants at different intervals following inoculation, would affect the infectivity of the virus. The entire leaves of 34 sets of 1 or more plants each of *Nicotiana glutinosa* and *Phaseolus vulgaris* were in-

oculated by rubbing with a purified preparation of tobacco-mosaic virus. The inoculated leaves were then washed off in running tap water, with the exception of the leaves of two sets of plants which were not washed. Instead of using the half-leaf method, 1 leaf of a plant of *P. vulgaris* was treated and the other leaf of the same plant served as a control. In the case of *N. glutinosa* 2 or 3 leaves were treated and 2 or 3 similar leaves of

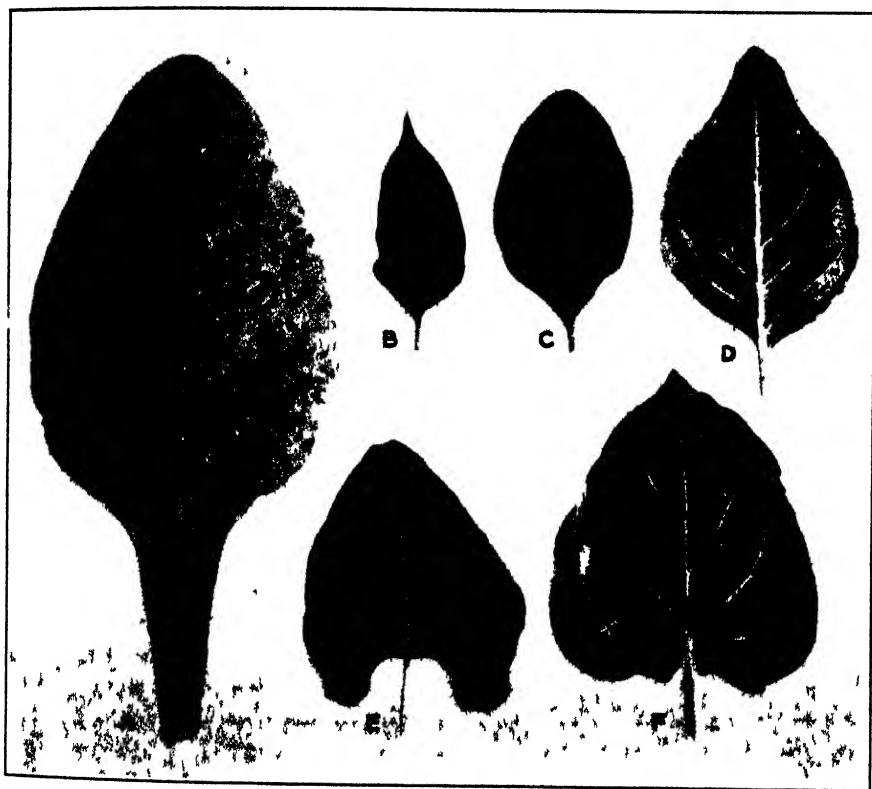


FIG 3 Leaves of (A) *Nicotiana glauca* Schrank, (B) *Capsicum frutescens* L., (C) *N. tabacum* L var *purpurea* x *glutinosa*, (D) *N. acuminata* Hook., (E) *Phaseolus vulgaris* L var Early Golden Cluster, and (F) *N. glutinosa* L as they appeared 5 days after inoculation. The right halves of the leaves were inoculated with tobacco mosaic virus and the left halves with another portion of the same tobacco mosaic virus preparation immediately after the addition of 0.5 mg of trypsin nitrogen per cc. The leaves are not susceptible to virus in the presence of trypsin as is shown by the absence of lesions in the portions of the leaves inoculated with virus plus trypsin.

the same plant were used as controls. Therefore, in one set, one leaf of each plant of *P. vulgaris* was rubbed immediately with a trypsin solution containing 0.5 mg trypsin nitrogen per cc., and the other leaf of the same plant was rubbed with distilled water. In the case of *N. glutinosa* 2 or 3

TABLE 8.—Effect of rubbing or spraying trypsin at various time intervals after inoculation of virus

Time interval in min., between inoculation of virus and administration of trypsin	Trypsin added by rubbing				Trypsin added by spraying			
	P. vulgaris		N. glutinosa		P. vulgaris		N. glutinosa	
	Control	Trypsin treated	Trypsin ÷ Control	Control	Control	Trypsin treated	Trypsin ÷ Control	Control
Immediately	31 <sup>a</sup>	2 <sup>a</sup>	6 <sup>b</sup>	137 <sup>a</sup>	41 <sup>a</sup>	2 <sup>a</sup>	5 <sup>b</sup>	38 <sup>a</sup>
5	47	7	15	50	64	4	6	150
10	33	3	9	61	82	5	6	235
20	60	7	12	42	42	17	40	41
30	94	40	43	23	217	112	52	119
40	204	144	70	84	129	97	75	133
60	129	76	59	83	150	115	76	237
75	121	90	74	92	100	58	58	187
90	124	101	81	65	153	129	84	202
110	138	137	99	108	137	118	86	300
120	153	137	90	225	170	125	73	105
150	150	124	83	50	163	140	86	226
180	209	192	92	78	130	114	88	191
240	163	120	74	40	129	115	89	225
300	183	171	94	103	109	97	89	325
420	166	158	95	113	229	210	92	234
1 day	200	181	90	260				

<sup>a</sup> Numbers represent the average number of lesions on 5 or more leaves of *P. vulgaris* var. Early Golden Cluster or 2 or more leaves of *N. glutinosa*.

<sup>b</sup> Numbers represent the average number of lesions per leaf on the trypsin-treated leaves divided by the average number of lesions per leaf on the corresponding control leaves multiplied by 100.



leaves were treated with the trypsin solution and 2 or 3 similar leaves of the same plant were rubbed with distilled water. This was repeated with the other set except that trypsin and water were administered by spraying from an atomizer instead of by rubbing. The leaves were then washed off in running tap water. The rest of the inoculated *P. vulgaris* and *N. glutinosa* plants, which had been washed in running tap water, were divided into 32 similar sets. At various intervals of from 5 minutes to 1 day after inoculation with virus, sets of plants were similarly treated by rubbing and by spraying with a trypsin solution and with distilled water. At the end of 5 days the average number of lesions on the trypsin-treated leaves was compared with the average number of lesions on the control or water-treated leaves. The results are given in table 8. The numbers in the columns designated by "Trypsin  $\div$  Control" represent the average number of lesions per leaf on the trypsin-treated leaves divided by the average number of lesions per leaf on the control leaves multiplied by 100, and hence may be regarded as a measure of the inhibitory effect of trypsin. It may be seen that trypsin was most effective on *P. vulgaris* during the first 30 minutes, at which time an abrupt decrease in inhibitory effect occurred, followed by a further gradual decrease until there was practically no effect. The effect on *N. glutinosa* was not so pronounced during the first 30 minutes and the subsequent decrease in the inhibitory effect was fairly gradual.

It was then decided to determine if spraying the trypsin solution on uninoculated leaves would have an effect on the subsequent inoculation of the leaves with virus. Accordingly, in this experiment, the leaves of 18 *Phaseolus vulgaris* plants and 8 *Nicotiana glutinosa* plants were sprayed with water, and the leaves of a like number of plants were sprayed with a solution prepared by dissolving 1 gm. of commercial trypsin in 50 cc. of water and adjusting to pH 6. The plants were watered from below so as not to wet the leaves until after the inoculation of the plants with virus. Six trypsin-sprayed *P. vulgaris* and 2 trypsin-sprayed *N. glutinosa* plants, and a like number of the water-sprayed plants, were then inoculated with a virus solution after 2 days, and similar sets were inoculated at the end of 4 and 6 days. The results as given in table 9 show that the sprayed trypsin caused a large decrease in the number of lesions. No lesions whatsoever appeared on the trypsin-sprayed leaves of *P. vulgaris*, even when there was a 6-day interval between spraying of trypsin and inoculation of virus. There was some tendency for the number of lesions on the trypsin-sprayed *N. glutinosa* leaves to increase as the time period was lengthened from 2 to 6 days.

Since no lesions appeared on the trypsin-sprayed leaves of *Phaseolus vulgaris*, it seemed desirable to determine if infection of Turkish tobacco plants with tobacco-mosaic virus could be prevented by a preliminary

TABLE 9.—*Effect of trypsin when sprayed on leaves before inoculation of virus*

Inoculated after →		2 days	4 days	6 days
Plants sprayed with water	<i>P. vulgaris</i>	45.0 <sup>a</sup>	46.2	38.7
	<i>N. glutinosa</i>	112.5	63.0	43.0
Plants sprayed with a trypsin solution	<i>P. vulgaris</i>	0.0	0.0	0.0
	<i>N. glutinosa</i>	5.3	8.5	15.0

<sup>a</sup> Numbers represent the average number of lesions on 12 leaves of *P. vulgaris* var. Early Golden Cluster or 10 leaves of *N. glutinosa*.

spraying of the tobacco plants with a solution of trypsin. In the previous experiment the trypsin-sprayed leaves were not watered by sprinkling from above until after the inoculation with virus, so in this experiment it was decided to determine the effect of watering. Accordingly, 20 flats of small Turkish tobacco plants containing 48 plants in each flat were divided into 4 groups of 5 flats each. One group was left untreated and was watered by sprinkling from above. The second group was left untreated and was watered from below, so as not to wet the leaves. The third group was sprayed thoroughly with a trypsin solution similar to that used in the preceding experiment and then watered by sprinkling from above. The last group was sprayed with trypsin, but was watered from below, so as not

TABLE 10.—*Infection of trypsin-treated Turkish tobacco plants with tobacco-mosaic*

Inoculation after →	1 day	2 days	3 days	4 days	5 da
(Untreated) watered from below ....	100.0 <sup>a</sup>	96.0	83.3	87.3	93.0
(Untreated) watered from above ....	98.0	91.6	81.2	83.0	98.0
Sprayed with trypsin and watered from below .....	0.0 <sup>b</sup>	4.2 <sup>b</sup>	4.2 <sup>c</sup>	6.2	14.0
Sprayed with trypsin and watered from above .....	12.5 <sup>b</sup>	29.1 <sup>c</sup>	62.5	87.5	81.5

<sup>a</sup> Numbers represent the per cent of infected plants out of 48 inoculated.

<sup>b</sup> After 10 days the diseased plants were removed and the healthy plants were re-inoculated and the percentage infection was 83.2, 82.5 and 90.5, respectively.

<sup>c</sup> After 10 days the diseased plants were removed and the healthy plants left uninoculated. All remained healthy.

to wet the leaves. One flat from each of the 4 groups was inoculated by rubbing 1 leaf of each plant once with infectious juice diluted 1-1000 with water 1 day after the spraying treatment. This was repeated after 2, 3, 4 and 5 days, using 1 flat from each of the 4 groups each day. Table 10 gives the percentage infection in each flat caused by this treatment. A high percentage of infection occurred in every flat that had not been treated with trypsin, whereas the infection was low in the flats that were inoculated 1 day after being sprayed with trypsin. The percentage infection rose as the interval between spraying and inoculation was increased, especially in the case of the flats watered by sprinkling from above. In the latter case the percentage infection was considerably higher at every time interval, and after 4 or 5 days it was comparable to that of the non-trypsin-treated controls. After 10 days a number of the healthy plants were reinoculated with virus and a high percentage of infection resulted, indicating that the trypsin treatment failed to produce any permanent protection. The results indicate that trypsin is lost from the leaves very slowly if the leaves are not washed, but that it is lost fairly rapidly if the leaves are washed. Furthermore, the trypsin must be present on the leaves at the time of inoculation of the virus or shortly thereafter in order to manifest its inhibitory action.

In order to obtain more definite information, it was decided to rub a trypsin solution over the leaves of *Nicotiana glutinosa* and *Phaseolus vulgaris* plants and test for the presence of trypsin on these leaves after time intervals, during which one set had been washed and watered as usual and the other set left unwashed and unwatered, except from below, so as not to wet the leaves. The tests for the presence of trypsin were made by inoculating sets of the plants with virus after the various time intervals and also by testing either the wash water from the leaves or an extract of the ground-up leaves for tryptic activity and for inhibitory action when added to a virus solution.

The leaves of 36 *Phaseolus vulgaris* plants and 6 *Nicotiana glutinosa* plants were rubbed 5 times each with a piece of gauze saturated with water. These plants, which were watered by sprinkling from above, served as controls and are designated as "Lot C." The leaves of 108 *P. vulgaris* plants and 18 *N. glutinosa* plants were rubbed 5 times each with a piece of gauze saturated with a solution prepared by dissolving 1 gm. of commercial trypsin in 50 cc. of distilled water and adjusting to pH 6. The leaves of half of these plants were then washed thoroughly in running water and watered from above. These washed and watered plants are designated as "Lot A." The leaves of the other half of the plants were not washed, the plants being watered from below. The plants from which the trypsin solution was not washed are designated as "Lot B." Four hours after the above de-

scribed treatment with water or a trypsin solution, 8 *P. vulgaris* plants and 2 *N. glutinosa* plants from each of lots A, B and C were inoculated with the same virus solution. This inoculation with virus was repeated on additional plants of lots A, B and C, 24 and 48 hours after the first treatment with water or trypsin. The average number of lesions appearing on 1 leaf from each of the 3 lots inoculated on the 3 successive days is given in table 11.

TABLE 11.—*Effect of washing trypsin-treated leaves*

Inoculated with virus →		After 4 hours	After 24 hours	After 48 hours
Lot A, plus trypsin (washed)	<i>P. vulgaris</i>	0.2 <sup>a</sup>	0.2	3.4
	<i>N. glutinosa</i>	23.6	42.1	64.4
Lot B, plus trypsin (unwashed)	<i>P. vulgaris</i>	0.0	0.0	0.1
	<i>N. glutinosa</i>	14.1	29.4	35.3
Lot C, control	<i>P. vulgaris</i>	77.0	164.0	142.0
	<i>N. glutinosa</i>	117.5	85.4	81.0

<sup>a</sup> The numbers represent the average number of lesions on 16 leaves of *P. vulgaris* var. Early Golden Cluster or 10 leaves of *N. glutinosa*.

The results show that a preliminary treatment of the leaves with trypsin either 4, 24, or 48 hours before the inoculation of the leaves with virus caused a marked decrease in the number of lesions produced by the virus. The shorter the time elapsing between the trypsin treatment and the inoculation, the greater was the effect produced by the trypsin. More lesions appeared after the 48-hour time period than after either the 24- or 4-hour period. The increase in the number of lesions as the period of time was lengthened indicates that the effective trypsin was being removed or destroyed slowly. The fact that more lesions were produced on the washed and watered leaves than on the unwashed leaves indicates that washing does remove some, but not all, of the trypsin from the leaves.

It seemed of interest to determine whether or not the trypsin actually remains on the leaves, and if so, whether or not it is in an active condition, and also to gain some idea of the location of such trypsin. Accordingly the leaves from 18 *Phaseolus vulgaris* plants and 3 *Nicotiana glutinosa* plants from lots A and B were used to prepare aqueous extracts by 3 different extraction procedures 24 hours after the trypsin treatment. Procedure No. 1 consisted of rubbing the surface of the leaves under a little water thoroughly with a small piece of gauze. It was hoped that this would remove all the trypsin which was on the surface of the leaves. Procedure No. 2 consisted of applying vacuum and then pressure to leaves placed first

under water and then out of water. It was hoped that this treatment would remove trypsin from the intercellular spaces and hair cells. The last procedure, No. 3, consisted of grinding up the entire leaf and preparing an aqueous extract from the macerated plant tissue in the expectation of obtaining all the trypsin from the leaves, regardless of its location. The amount of trypsin present in each of these 12 extracts was then determined by testing directly for tryptic activity by the formol titration and also by determining the reduction in the number of lesions produced when 5 cc. of each of the extracts was added to 5 cc. of a sample of virus and inoculated on leaf-halves against a control consisting of the virus diluted with an equal volume of water.

A comparison of the results obtained by the 2 methods of testing was desirable, hence table 12 was arranged to give the results of each test on a

TABLE 12.—*Comparison of chemical and plant tests for trypsin*

Extract from ↓	Extraction method	Lot A		Lot B	
		Formol <sup>a</sup> titration	Lesions on <sup>b</sup> <i>P. vulgaris</i>	Formol <sup>a</sup> titration	Lesions on <sup>b</sup> <i>P. vulgaris</i>
<i>P. vulgaris</i> .....	1	0.0	1.5	34.8	48.5
<i>N. glutinosa</i> .....	1	0.0	0.0	69.5	70.0
<i>P. vulgaris</i> .....	2	8.7	25.0	52.1	63.8
<i>N. glutinosa</i> .....	2	8.7	46.5	26.1	61.2
<i>P. vulgaris</i> .....	3	8.7	20.5	100.0	94.4
<i>N. glutinosa</i> .....	3	26.1	51.0	82.5	96.3

<sup>a</sup> Numbers directly proportional to the number of cc. of 0.02 N NaOH used.

<sup>b</sup> Numbers inversely proportional to the actual number of lesions.

percentage basis, the extract requiring the greatest amount of 0.02 N sodium hydroxide in the formol titration being listed as 100.0 and the remaining titrations being based on this number. The more trypsin present, the greater is the amount of sodium hydroxide needed in the formol titration and the larger the number in the table; in the test on *Phaseolus vulgaris* the more trypsin present, the fewer are the number of lesions, thus, in this case, the numbers are inversely proportional to the number of lesions. It may be seen that, in general, the results obtained by the formol titration method for trypsin are in fairly close agreement with those obtained by testing the solutions for their effect on the infectivity of the virus. The results also indicate that much active trypsin remained on the surface of the leaf when it was not washed, but that washing the leaves or watering the plants by sprinkling removed nearly all of this trypsin. Furthermore, after the trypsin had been removed by simple washing there remained ac-

tive trypsin on or in the leaf that could not be rubbed or washed off, the presence of which could be demonstrated by applying pressure and vacuum to remove it from the leaf or by thoroughly macerating the leaf and then pressing out the liquid. It has been demonstrated, therefore, that trypsin sprayed on a leaf exerts its inhibitory effect for periods as long as one week if the leaf is not washed, but that such trypsin can be removed by thorough washing and does not interfere with a subsequent inoculation of virus. However, the treatment of a leaf with a trypsin solution by rubbing fixes the trypsin in or on the leaf, so that some of the trypsin remains on the leaf and cannot be removed without destroying the leaf. This trypsin interferes with a subsequent inoculation of the leaf with virus.

*The Effect of Trypsin on the Susceptibility of Plants to Different Viruses.* Since the inhibitory effect of trypsin on the susceptibility of plants to tobacco-mosaic virus has been demonstrated, using many different species of plants as hosts, and since other experiments indicate that this effect is due to the action of trypsin on the host plant, attempts were made to demonstrate this inhibitory effect with different strains of tobacco-mosaic virus and with entirely different viruses. The viruses used in these experiments were severe etch, tobacco ring-spot, ordinary cucumber mosaic, a yellow strain<sup>3</sup> of cucumber-mosaic (10), aucuba-mosaic, and two other widely differing strains of tobacco-mosaic, namely a masked (3) and a yellow (4) strain.<sup>4</sup> All viruses were obtained from infected Turkish tobacco plants, except aucuba-mosaic virus, which was from infected tomato plants. The masked tobacco-mosaic, yellow tobacco-mosaic, and aucuba-mosaic viruses were tested on leaf-halves of *Phaseolus vulgaris* and *Nicotiana glutinosa*. Samples of untreated infectious juice plus an equal volume of 0.01 N HCl were used as controls and tested against similar samples of infectious juice plus an equal volume of 0.01 N HCl containing varying amounts of trypsin. The final trypsin-treated juice samples contained 0.5, 0.1 or 0.01 mg. trypsin nitrogen per cc. Severe etch was tested on Turkish tobacco, ring-spot on cowpea, *Vigna sinensis* Endl. var. Black Eye, and the two strains of cucumber-mosaic virus were tested on both Turkish tobacco and *V. sinensis*. The tests on Turkish tobacco were made by inoculating one flat of 48 plants by rubbing one leaf of each plant once with gauze dipped in infectious juice diluted 1 to 50 with water and a second flat with a similar sample of juice diluted 1 to 50 with water and containing 0.1 mg. trypsin nitrogen per cc. The tests on *V. sinensis* were made by inoculating 15 or more plants by rubbing the leaves with gauze dipped in infectious juice and 3 similar sets of plants with 3 additional samples of the same infectious

<sup>3</sup> Designated by Dr. W. C. Price as strain No. 6.

<sup>4</sup> This virus produces large, sharply defined, yellow primary lesions, and yellow mosaic mottling and distortion on systemically infected leaves of Turkish tobacco plants.

juice containing 0.5, 0.1 and 0.01 mg. trypsin nitrogen per cc., respectively. The average number of lesions per half-leaf or per plant obtained on plants of *P. vulgaris*, *N. glutinosa* and *V. sinensis* with controls or nontrypsin-treated samples of infectious juice was compared with the average number of lesions obtained with the corresponding trypsin-treated samples of infectious juice. When Turkish tobacco was used as the test plant the percentage of infected plants obtained upon inoculation of untreated juice was

TABLE 13.—*Effect of different amounts of trypsin on the susceptibility of plants to different viruses*

Milligrams trypsin nitro- gen per cc. →	0.5			0.1		0.01	
	Test plant	Control	Trypsin	Control	Trypsin	Control	Trypsin
Tobacco mosaic (yellow strain)	<i>P. vulgaris</i>	144 <sup>a</sup>	0	187	0.5	206	1.5
	<i>N. glutinosa</i>	157 <sup>a</sup>	8	202	30	206	92
Tobacco mosaic (masked strain)	<i>P. vulgaris</i>	88 <sup>a</sup>	0	148	0.3	100	1.1
	<i>N. glutinosa</i>	225 <sup>a</sup>	12	185	26	153	108
Aucuba mosaic	<i>P. vulgaris</i>	23 <sup>a</sup>	0	17	0	7	0.5
	<i>N. glutinosa</i>	95 <sup>a</sup>	3	38	10	35	26
Cucumber mosaic (Ordinary)	<i>V. sinensis</i>	8.8 <sup>b</sup>	0	8.8	0	8.8	0
	Turkish			85 <sup>c</sup>	0		
Cucumber mosaic (yellow strain)	<i>V. sinensis</i>	5.4 <sup>b</sup>	0	5.4	0	5.4	0
	Turkish			34 <sup>c</sup>	0		
Severe etch	Turkish			77 <sup>c</sup>	0		
Tobacco ring-spot	<i>V. sinensis</i>	9.4 <sup>b</sup>	0	9.4	0	9.4	0

<sup>a</sup> Numbers represent the average number of lesions on 12 half-leaves of *P. vulgaris* var. Early Golden Cluster, or 10 half-leaves of *N. glutinosa*.

<sup>b</sup> Numbers represent the average number of lesions on 15 or more plants of *V. sinensis*.

<sup>c</sup> Numbers represent the percentage of infected plants out of 48 Turkish tobacco plants.

compared with the percentage of infected plants obtained upon inoculation of the corresponding trypsin-treated preparation. The results (Table 13) show that a marked reduction in susceptibility of plants occurred upon addition of trypsin, regardless of the virus used. The results also indicate that, in general, larger amounts of trypsin cause plants to be less susceptible to the virus. The difference in response of *N. glutinosa* and *P. vulgaris* to the same trypsin-virus sample, which has been mentioned previously, is again evident. For example, a sample of masked strain of tobacco-mosaic virus plus 0.01 mg. trypsin nitrogen per cc., which gave an average of 108 lesions per half-leaf on *N. glutinosa*, gave only 1.1 lesions per half-leaf on *P. vulgaris*. This same virus sample plus 0.5 mg. trypsin nitrogen per cc. gave 12 lesions on *N. glutinosa* and no lesions on *P. vulgaris*. It has been demonstrated, therefore, that plants, susceptible to one or more of two widely differing strains of tobacco-mosaic, aucuba-mosaic, two strains of cucumber-mosaic, tobacco ring-spot, and severe etch, become much less susceptible when virus is inoculated in the presence of trypsin.

*The Rate of Diffusion of Trypsin in the Presence of Tobacco-mosaic Virus.* Most of the evidence from the experiments described in the foregoing paragraphs has indicated that trypsin affects the test plant used to measure the infectivity of the virus. No definite evidence that trypsin affects the virus has been obtained, although some of the results could be interpreted on this basis. Since it has been demonstrated that trypsin does not inactivate the virus by its hydrolytic action, any direct inactivating action of trypsin would suggest an inactive combination or association complex of virus with trypsin. The isoelectric point of the virus is not known with certainty, although some investigators (13) consider that it is negatively charged in the range above about pH 3. If the virus does possess a negative charge above pH 3 it does not appear impossible that inactivation might be brought about on the formation of a complex by the union of oppositely charged units, since proteins having a high isoelectric point are positively charged in this range. In such a case the complex would be different from a simple adsorption complex, since when activated charcoal in the proper amount is added to a virus solution the apparent infectivity increases, yet it may be demonstrated that most of the virus is adsorbed on the charcoal. It appears that something other than simple adsorption is necessary to account for loss of virus infectivity, yet the reaction cannot be too involved, since the virus infectivity may be regained by the removal of trypsin.

Perhaps the simplest example of a phenomenon that might be regarded as analogous is the reaction between heme and globin. In this case heme is not active when free and gains its physiological activity only when in combination with globin, a protein having an isoelectric point above pH 7.



This combination of heme with globin is readily reversible. If there is a similar combination of virus with trypsin, the properties of trypsin in combination should be different from the properties of free or ordinary trypsin. It would be expected that the rate of diffusion of a virus-trypsin complex would be different from the rate of diffusion of trypsin. It was decided, therefore, to determine the rate of diffusion of trypsin in the presence of tobacco-mosaic virus. Since the size of the virus is not known with certainty and since nothing is known concerning the probable ratio of virus to trypsin in such a complex, it is impossible to be certain that any given virus preparation contains enough virus to combine with all of the trypsin molecules in a solution containing but a few milligrams of trypsin nitrogen per cubic centimeter. It is necessary, of course, that most of the trypsin be in combination if a difference in the rate of diffusion is to be found. Although it would be impossible to be certain that all or most of the trypsin molecules have gone into combination with virus, the most favorable experimental conditions would consist of a very dilute trypsin solution plus a very concentrated virus solution. However, for the diffusion experiments, which were performed using a Northrop-Anson (7) diffusion cell, it was necessary to have sufficient trypsin present so that the amount diffusing represented a measurable amount and but a very small percentage of the total trypsin present in the cell. Nevertheless, it was possible to drop the trypsin concentration to about 1 mg. of trypsin nitrogen per cubic centimeter. A concentrated purified virus preparation capable of producing lesions on *Phaseolus vulgaris* when diluted  $10^{-8}$  was used.

The rates of diffusion were determined by the method described by Northrop and Anson (7) and used by Scherp (12) to determine the diffusion coefficient of crystalline trypsin. A solution of crystalline trypsin in 0.5 saturated magnesium sulphate plus 0.1 M sodium acetate at pH 4 containing about 2 mg. of trypsin nitrogen per cc. was prepared. To one volume of this solution was added an equal volume of the concentrated purified virus preparation in 0.1 M phosphate at pH 4. To another volume of the trypsin solution was added an equal volume of 0.1 M phosphate at pH 4. Outside the cell at the start of the experiments was a solution at pH 4, containing 0.25 saturated magnesium sulphate, 0.05 M sodium acetate and 0.05 M phosphate. The cell was first filled with the solution containing trypsin, but no virus, and the amount of trypsin diffusing across the glass membrane in successive 18-hour intervals was determined.<sup>5</sup> The cell was then filled with the solution containing trypsin plus virus and the amount of trypsin diffusing across the glass membrane in successive 18-hour intervals was again determined. The amount of trypsin diffusing across

<sup>5</sup> The trypsin determinations were made by Dr. M. Kunitz and Miss Margaret MacDonald.

the membrane from the trypsin solution in a given time interval was found to be the same as the amount of trypsin diffusing from the trypsin plus virus solution. Therefore, the rate of diffusion of trypsin is not changed by the presence of virus at the above mentioned concentration. This may be regarded as an indication that a combination of virus with trypsin does not occur, since the experimental conditions were most favorable for the combination. However, it cannot be considered absolute proof of the absence of such combination because of the lack of information concerning the size of the virus particle, the maximum number of particles present in a given virus solution and the probable ratio of virus to trypsin in such a complex.

#### DISCUSSION

The evidence obtained in this investigation indicates that the loss of infectivity of the virus of tobacco mosaic upon the addition of trypsin is not due to the proteolytic action of the enzyme. Tryptic hydrolysis is a time reaction which takes place rapidly only within a definite and fairly narrow range of hydrogen-ion concentration, whereas the loss of infectivity of the virus takes place immediately and over a wide range of hydrogen-ion concentration. Resynthesis or reactivation of the virus would not be expected if it were actually hydrolyzed, yet its infectivity in a trypsin-virus solution was regained by such simple procedures as dilution, heating, or removal of the trypsin from the solution by self-digestion or by digestion with pepsin. The fact that nonproteolytic substances, such as globin and trypsinogen, reduce the infectivity of the virus suggests that the reduction in the case of trypsin may not be due to proteolysis. No evidence from these experiments indicates that the virus of tobacco mosaic is a protein.

It was found possible to demonstrate the loss of infectivity of virus on the addition of trypsin, using several different viruses, and measuring the loss on many different species of plants. The fact that the rate of diffusion of trypsin was found to be unchanged in the presence of a fair concentration of virus is an indication that trypsin does not cause the loss of infectivity by forming an inactive complex with virus. Evidence that trypsin affects the plant and that the degree of its action on plants may vary from one species to another, was obtained when it was found possible to prepare a trypsin-virus solution that produced lesions on *Nicotiana glutinosa* and yet failed to produce lesions on *Phaseolus vulgaris*. Similar evidence was obtained in experiments in which it was found that addition of small constant amounts of trypsin to dilutions of virus caused a fairly constant decrease in the number of lesions, at all dilutions, when tested on *N. glutinosa*. The difference in the response of *N. glutinosa* and *P.*

*vulgaris* to the same trypsin-virus solution, and the fact that the degree of inhibition was fairly constant on the former when the actual amount of virus present was varied widely, is additional evidence that trypsin affects the plant. It is concluded, therefore, that the effect of trypsin in causing a loss in virus infectivity is due chiefly to its action on the plant. This conclusion is at variance with that of Caldwell (1) who reported that he found no action of trypsin on the tissues of the host plant. He found the average number of lesions, on leaves of *N. glutinosa* inoculated with virus, to be about the same, whether or not the leaves were rubbed with a trypsin solution previous to inoculation. In our experiments, which have been repeated several times, preliminary treatment with trypsin, either by rubbing or spraying, has always resulted in a marked decrease in the average number of lesions on inoculation with virus.

The action of trypsin on the test plant was found to be temporary, since the inhibitory effect of trypsin, which had been sprayed on a plant, was eliminated almost entirely by thoroughly washing the plant before inoculation with virus. Washing also decreased markedly the effect of trypsin that had been rubbed over a leaf. All the results indicate that trypsin must be present with the virus, either on the leaf at the time of inoculation, or within about thirty minutes thereafter in order to produce its inhibitory effect. Since it appears that this effect is caused by the action of trypsin on the plant, the infected cells seem to require at least 30 minutes to heal or transmit the virus to an adjacent uninjured cell, into which it may be assumed trypsin does not enter. If trypsin is present at the time of inoculation or within 30 minutes thereafter, it apparently affects infected cells, possibly by killing them, so that the virus does not spread and infect the plant. The results indicate, therefore, that the establishment of virus in a plant is not a rapid process, since infection may be inhibited by subsequent treatment. It appears to be, on the contrary, a time process requiring at least 30 minutes for completion.

#### SUMMARY

The decrease in infectivity of the virus of tobacco mosaic on addition of trypsin, as measured on *Nicotiana glutinosa* L. and *Phaseolus vulgaris* L., is not due to the proteolytic activity of the trypsin, because (1) the loss in infectivity is immediate, (2) it may take place over a wide range of hydrogen-ion concentrations, including some at which trypsin is inactive proteolytically, and (3) the infectivity of the virus may be regained by heat, by dilution, or by digestion and removal of trypsin.

A similar loss of the infectivity takes place on addition of trypsinogen or globin, proteins, which possess no proteolytic activity, but which, like trypsin, have isoelectric points above pH 7.

Virus, sufficiently concentrated so that it produces lesions on *Phaseolus vulgaris* when diluted  $10^{-8}$ , does not affect the rate of diffusion of trypsin at a concentration of about 1 mg. of trypsin nitrogen per cubic centimeter. This indicates that there is no combination of virus with trypsin.

It is possible to prepare a trypsin-virus solution that will produce no lesions on plants of *Phaseolus vulgaris*, but will produce many lesions when tested on plants of *Nicotiana glutinosa* having about an equal susceptibility to untreated virus. This demonstrates that trypsin affects, in some way, the cells of plants of *P. vulgaris*.

The addition of a small constant amount of trypsin to dilutions of tobacco-mosaic virus causes a fairly uniform reduction in the number of lesions on *N. glutinosa* at such dilutions.

The decrease of the infectivity of this virus on addition of trypsin has been demonstrated upon a number of different species of plants including *Nicotiana glutinosa*, *N. acuminata* Hook., *N. langsdorffii* Schrank, *N. rustica* L. var. Winnebago, *N. tabacum* L. var. *purpurea*  $\times$  *glutinosa*, *N. tabacum* var. Turkish, *Datura stramonium* L., *Phaseolus vulgaris* var. Early Golden Cluster, *Physalis angulata* L., *Solanum melongena* L. var. Black Beauty, *S. pseudo-capsicum* L., and *Capsicum frutescens* L.

Other viruses whose action is similarly affected by trypsin are a masked and a yellow strain of tobacco mosaic, aucuba mosaic, severe etch, tobacco ring spot, ordinary cucumber mosaic, and a yellow strain of cucumber-mosaic virus.

Trypsin sprayed or rubbed on the leaves of plants of *Nicotiana glutinosa*, *N. tabacum* var. Turkish, and *Phaseolus vulgaris* markedly lowers their susceptibility to virus. This effect is temporary, however, since the plants become susceptible to the virus again upon washing trypsin from the leaves with water.

The inhibitory effect of trypsin may be demonstrated if it is administered by either rubbing or spraying within about 30 minutes after the inoculation of the leaf with virus. This indicates that the establishment of virus in a plant, so that it may not be interrupted by subsequent treatment, is not instantaneous, but requires at least 30 minutes for completion.

As a whole, the results indicate that the loss of infectivity upon the addition of trypsin to a virus solution is due chiefly to the action of trypsin on the plant.

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# THE WEDGE GRAFT AS A MEANS OF CONTROLLING OVERGROWTHS AT THE UNION OF NURSERY APPLE TREES<sup>1</sup>

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When it was established through the work of Riker and Keitt (5, 6) and Muncie (3) that about 95 per cent of the overgrowths at the union of grafted apple trees is not due to *Pseudomonas tumefaciens* E. F. Sm. and Town., the problem of controlling these malformations became simplified. Riker and Muncie (8) showed that well-matched and fitted grafts developed less callus knot than poorly fitted ones. In 1928, Melhus, Muncie, and Fisk (2) suggested the wedge graft as being beneficial in the control of callus knot. In field trials the same year Muncie (4) obtained an increase of about 20 per cent in clean trees by the use of the wedge graft. Later work by Riker, Keitt, and Banfield (7) showed that a light grade of adhesive plaster or nurseryman's tape applied as a wrapper at the union of apple grafts also reduced the amount of callus knot by about 20 per cent. In 1929 Maney (1) reported that the double tongue graft controlled callus knot on Sharon apple.

In view of the new understanding referred to above regarding the causation of overgrowths at the union of apple grafts, coupled with the findings of Melhus, Muncie, and Fisk (2) and Muncie (4), that wedge grafts showed some tendency to reduce the amount of callus knot, a careful study of the comparative value of the different types of grafts from the standpoint of stand of trees, strength of graft unions, and location of and freedom from callus knot was warranted.

## METHODS AND MATERIALS

The comparative value of wedge or cleft and single-tongue or whip grafts for the control of callus knot was studied in 7 different nurseries,<sup>2</sup> while the value of the double-tongue graft was studied in 2 of the nurseries.

<sup>1</sup> The writer wishes to acknowledge his indebtedness to Dr. I. E. Melhus of the Botany Department of Iowa State College for suggesting the problem and for criticism and suggestions received throughout the course of these investigations and the preparation of this manuscript. Credit also is due Dr. J. H. Muncie, now of the Botany Department of Michigan State College, for assistance in planning some of the field work.

<sup>2</sup> The writer is indebted to the following nurseries for excellent cooperation in the study of this problem: Mount Arbor Nurseries and Shenandoah Nurseries, Shenandoah, Iowa; Plumfield Nurseries, Fremont, Nebraska; Marshall Nurseries, Arlington, Nebraska; J. H. Skinner and Company, Topeka, Kansas; Stark Bros. Nurseries, Stigler, Oklahoma; and Hillenmeyer Nurseries, Lexington, Kentucky.

Two of these nurseries are in Iowa, 2 in Nebraska, and 1 each in Kansas, Oklahoma, and Kentucky. In each case the plantings were made in the center of a large block of apple trees, so that the cultivation and treatment would be representative of the usual nursery practice. Scions of the Wealthy apple generally were used because of the susceptibility of this variety to callus-knot formation. However, several other varieties were included at various times.

The wedge graft is commonly used for top grafting and double working of apple trees, the side graft being a modified wedge graft. In piece-root grafting the wedge graft is easily made and requires one less cut than the whip graft. The usual procedure in making the wedge graft is somewhat as follows: The base of the scion is cut to a feather-edge wedge,  $\frac{3}{4}$  inch long, by 2 cuts, as shown in figure 1, A, *a*. In the case of the stock, a slightly cross-grained cleft is made to a depth of about  $\frac{1}{8}$  inch deeper than the length of the scion wedge (Fig. 1, A, *b*). The cleft is slightly cross-grained to prevent splitting. In making the graft, the wedge is fitted tightly into the cleft of the stock, so that the cut surface of the scion is completely covered by the stock. Figure 1, B and C, shows a properly fitted graft.

The cambium of the scion wedge should be matched along one side with that of the stock, if the scion is smaller in diameter. The graft may be wrapped with waxed string or with nurseryman's tape as recommended by Riker, Keitt, and Banfield (7). It often happens that the wedge graft is improperly made. Figure 1, D, shows 2 common errors. In the one the scion wedge (*d*) is larger in diameter than the stock, which allows the cambium along one side of the wedge and the scion tip to project beyond the stock and thus give favorable conditions for callus-knot development, as reported by Riker and Muncie (8) and Sass (9). In the other the cut surface (*e*) of the scion is not completely covered by the stock, and knots may develop from this exposed cut surface.

The double-tongue graft recommended by Maney (1) is similar to the whip or single-tongue graft. The tip of the scion lip is wedged by removing about  $\frac{1}{4}$  inch of bark and wood from the outer side. This wedged scion lip is inserted into a second cut that is made in the stock. This gives 2 tongues fitting into the stock, hence the name double-tongue graft. Two more cuts are required for this graft than for the whip graft.

The grafts used in the experimental plots were made at each nursery where the plots were located. The making of the grafts was supervised by J. H. Muncie or by the writer. In planning an experiment, an equal number of both wedge and whip grafts was used for each variety. After these grafts were made they were stored and planted in accordance with the established practice in that particular nursery. No special care was given the

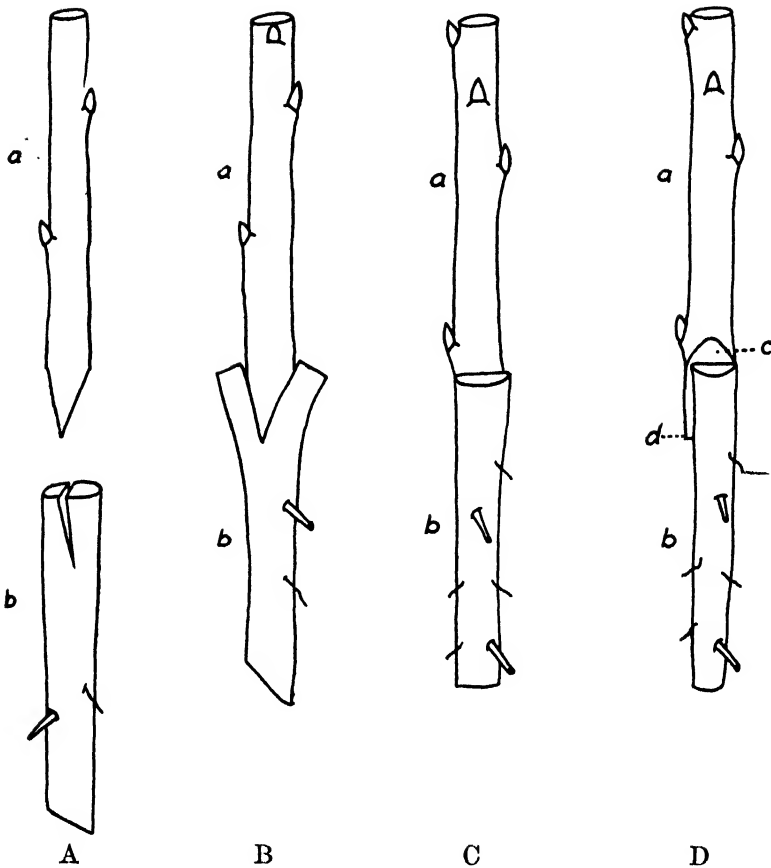


FIG. 1. A-D. The wedge or cleft graft, as used in piece-root grafting. In D, *c* and *d*, are shown two common errors in fitting.

experimental grafts throughout the growing season. Final examination of the grafts was made at digging time, when the trees were 2 years old. In several cases the grafts were examined when the trees were only a year old.

#### A COMPARISON OF THE RIGIDITY OF THREE TYPES OF GRAFTS

Rigidity in apple grafts is important in nursery practice, especially with reference to handling, planting, and various methods of cultivation. To determine, if possible, the relation between the strength of the different types of grafts, 600 Wealthy apple grafts were obtained from each of 3 nurseries. These grafts were of the 3 types, wedge, whip, and double-tongue. An equal number of each type of graft was wrapped with waxed string and nurseryman's tape, following which all grafts were allowed to callus for a month. The rigidity of the union of the grafts was determined



when held in different positions. The graft was suspended by supporting it 1 inch on either side of the union, while the force was applied at the union.

The apparatus<sup>3</sup> used for determining the rigidity of the grafts was devised for testing the strength of corn stalks. By means of the apparatus, breaking pressure was brought upon the graft; the stress or tension was recorded by means of a calibrated spring. The breaking force in pounds for the grafts of various sizes in the different lots is given in table 1. Each figure given is an average of the results from at least 10 grafts and, in many sizes, as many as 25 or 30. The findings show that more force was required to break grafts of greater diameter. An average of the measurements for each lot shows that the grafts wrapped with waxed string required about the same force for breaking, regardless of the type of graft. The grafts usually broke more readily parallel to the cut of the graft. The mechanical strength of grafts wrapped with nurseryman's tape was increased about 3 pounds. It is shown also that the uncut scion of the same diameter was stronger than the graft, whether wrapped with waxed string or with nurseryman's tape. These grafts were not made especially for this test and were the usual bench run at the nursery. In observing the individual grafts as they were broken it was seen that twice the force often was required to break the better made and fitted grafts as compared with the poorly fitted grafts of the same size.

#### THE STAND OF APPLE TREES OBTAINED FROM THREE TYPES OF GRAFTS

One of the important problems of the nurseryman is to obtain from the grafts planted a high percentage stand of clean trees. Table 2 shows the percentage of trees obtained after 2 seasons' growth, as well as the percentage of clean trees obtained from the original lot of 500 grafts in each case. These data are from trees dug from the experimental plots during the fall of 1930. Complete figures for 1928 and 1929 are not available, but, in general, agree with the results shown in table 2. Nearly all factors shown affect the stand of trees. The greatest difference in stand seems to be the result of location. Grafts grown in Kansas produced a much better stand than those in Iowa and Nebraska; but there is less difference between total trees and clean trees from grafts grown in Nebraska. The type of graft does not seem materially to affect the stand, although the wedge graft may show a slight advantage. Usually, the type of wrapper is not important with reference to stand; the variations one way or another are not constant.

While table 2 shows the final stand obtained from the growing of apple grafts, it does not indicate the reaction of the various types of grafts during

<sup>3</sup> The writer is indebted to Dr. L. W. Durrell, formerly of the Botany Department, Iowa State College, for the original design of the apparatus used.

TABLE 1.—A summary of the force in pounds necessary to break wedge, whip, and double-tongue-grafts when wrapped with waxed string or nurseryman's tape

Lot	Type of graft and treatment	Scion caliper in sixteenths of an inch																
		2-3		3-4		4-5		5-6		6-7		7-8		5-6		6-7		Av.
		2-3	3-4	4-5	5-6	3-4	4-5	5-6	6-7	4-5	5-6	6-7	7-8	5-6	6-7			
		Stock caliper in sixteenths of inches																
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Ia	Whip graft, string wrapped	5.5	9.2	7.5	10.4	12.1	15.0	10.7	17.9	12.6	18.5	12.0	18.0	12.4				
IIb	" " "	6.4	10.5	8.2	10.3	13.1		12.4	14.4	17.1	24.5	19.5	19.0	14.1				
IIIa	" " tape wrapped	5.5	7.4	8.9	11.5	13.1	14.5	21.8	23.7	25.2	22.0	29.0	16.8					
IVb	" " "	5.5	8.0	10.0	11.0	10.4	12.3	14.3	17.8	19.6	26.0	25.4	25.0	15.5				
Va	Wedge graft, string wrapped	6.0	6.1	6.3	8.2	10.5	11.4	11.6	13.3	14.7	17.5	21.0	19.5	12.2				
VIb	" " "	6.7	6.3	7.7	9.8	12.8	16.5	15.6	18.0	19.5	19.0	22.0	13.9					
VIIa	" " tape wrapped	7.6	8.7	9.5	8.9	12.4	13.6	17.2	16.5	21.0	24.5	28.5	29.0	29.0	17.4			
VIIIb	" " "	6.5	7.2	10.4	10.5	12.4	12.7	16.5	22.1	23.8	20.5	25.3	25.6	16.1				
IXa	Double-tongue graft, string wrapped	6.0	7.0	8.0	8.5	9.4	11.1	14.0	15.1	17.8	23.3	18.5	16.9	25.5	14.3			
Xb	" " "	6.1	7.3	7.1	9.7	11.4		12.8	13.5	15.5		16.3	20.2	12.4				
XIa	" " tape wrapped	6.2	7.6	8.5	8.9	10.5	11.9	17.0	17.3	19.3	21.2	24.5	26.8	15.9				
XIIb	" " "	6.0	7.3	9.9	11.3	13.4	12.05	14.6	18.5	21.5	20.4	24.2	15.7					
Scion		8.5					14.0	23.5				30.0	19.0					

<sup>a</sup> Grafts broken parallel to the cuts of the grafts.  
<sup>b</sup> Grafts broken at right angles to the cuts of the grafts.

TABLE 2.—Effect of type of graft and kind of wrapper on the stand of two-year-old apple trees

Variety	Location	Wedge graft						Whip graft					
		Tape wrap			String wrap			Tape wrap			String wrap		
		Stand	Clean trees	Per cent	Stand	Clean trees	Per cent	Stand	Clean trees	Per cent	Stand	Clean trees	Per cent
Wealthy	Iowa	94 <sup>a</sup>	93	62	59	87	85	82	82	78			
"	Nebraska	74	71	67	64	70	64	68	68	59			
"	Kansas	98	87	96	68	96	76	97	97	55			
Yellow transparent	Nebraska	67	64	79	76	82	75	76	76	68			
Yellow transparent	Kansas	99	88	99	68	96	80	94	94	65			
Whitney	Nebraska	78	78	78	76	63	58	76	76	62			

<sup>a</sup> Percentage of stand obtained from planting 500 grafts.

the first season's growth. For this work, each of the three types, whip, wedge, and double-tongue, were divided into 4 series as follows:

1. Wrapped with tape; scion  $\frac{1}{8}$  inch and less in diameter.
2. Wrapped with tape; scion greater than  $\frac{1}{8}$  inch in diameter
3. Wrapped with string; scion  $\frac{1}{8}$  inch and less in diameter.
4. Wrapped with string; scion greater than  $\frac{1}{8}$  inch in diameter.

These grafts were planted on April 25, 1930, in the experimental plots at Ames, Iowa. Stand counts were made each month throughout the summer, and on October 25 the trees were dug and examined. The summer of 1930 being extremely dry, the results obtained from this study would indicate the performance of the 3 types of grafts under adverse conditions. More favorable conditions were obtained in the plots in other localities the previous year, the results of which have been given. Table 3 shows the

TABLE 3.—*Results of stand counts of Wealthy apple grafts at intervals of one month throughout the summer of 1930*

Date counted	Double-tongue series				Whip series				Wedge series			
	1	2	3	4	1	2	3	4	1	2	3	4
4/25/30 .....	262	289	292	275	291	261	180	324	266	289	248	307
5/31/30 .....	223	244	259	241	247	184	157	300	157	222	208	268
6/30/30 .....	201	227	245	241	205	183	157	283	157	222	202	259
8/4/30 .....	161	171	183	175	191	183	141	255	138	174	181	236
9/8/30 .....	159	167	177	167	190	182	141	254	137	174	179	235
10/6/30 .....	157	165	177	167	185	179	141	252	137	174	177	235
Stand percentage of grafts planted .....	59.9	57.1	60.6	60.7	63.5	68.5	78.3	77.7	51.5	60.2	71.3	76.5
Percentage of final stand, clean trees..	86.6	84.2	61.0	47.8	83.7	89.3	60.9	49.2	92.7	92.5	73.4	74.8

stand throughout the summer as well as the number of clean trees obtained in each type of graft. The double-tongue graft shows the lowest final stand in all series, with the exception of series 1 of the wedge graft. When the percentage of clean trees produced is considered, the double-tongue graft was also the lowest. The type of wrapper did not materially affect the stand of the double-tongue grafts, although the grafts wrapped with tape gave a higher percentage of clean trees. The whip graft showed the highest percentage of stand, while the clean trees produced exceeded the double-tongue graft by at least 10 per cent in all but series 1. The grafts wrapped with tape gave a higher percentage of clean trees, although the string-wrapped grafts gave a higher final stand. The wedge graft was between the other 2 types of grafts in the stand produced. The percentage of clean trees produced from grafts wrapped with tape was lower in series 1 than

the other 2 types. In series 2 the percentage was above the double-tongue and below the whip graft. Those grafts wrapped with string showed a much higher percentage of clean trees than either of the other 2 types. As was found in the whip grafts, the wedge grafts wrapped with string showed a higher percentage of stand than those wrapped with tape.

In nursery practice the grafts usually are not counted when being planted. The percentage of clean trees is then calculated from the final stand. As shown in table 3, when the percentage of clean trees is calculated from the final stand, the wedge graft wrapped with tape are found to be over 90 per cent clean trees, while those grafts wrapped with string showed over 70 per cent of clean trees. In every case these percentages are higher than those obtained from the double-tongue or whip grafts, while in the case of the string-wrapped grafts the increase shown by the wedge graft is over 10 per cent in series 3 and over 20 per cent in series 4.

The size of the scion used for grafting did not materially affect the stand of trees produced, except in the wedge grafts, where the larger scions produced a higher percentage of trees. However, in the case of series 4 of the double-tongue and whip grafts, the smaller scions showed at least 10 per cent more clean trees than the grafts made with the larger scions. This fact was not apparent in the wedge grafts.

During the first month of the growing period the grafts are becoming established. Table 3 shows that the greatest loss in stand of wedge and whip grafts occurred during this period. A second rapid loss during the third month was shown by the double-tongue grafts. After the third month of growth there was practically no further loss in stand. During 1930 the third month, July, was extremely dry. This might account for the rapid drop during this period. Under normal conditions this second drop in stand might not occur and, after the initial loss, the stand would tend to remain constant.

#### RESULTS OF THE USE OF THE WEDGE GRAFT FOR THE CONTROL OF CALLUS KNOT

Preliminary trials by Melhus, Muncie, and Fisk (2) and Muncie (4) showed that the wedge graft was beneficial in the control of callus knot. Consequently, numerous experiments at the 7 nurseries were conducted in 1928, 1929, and 1930. A summary of the results obtained when wedge grafts of the different varieties of apple were made is shown in table 4. These results show that in every case a higher percentage of clean trees was obtained by using the wedge graft. The greatest increase in clean trees was obtained with Wealthy, Yellow Transparent, Sweet Russet, Whitney, and Duchess varieties. On the other hand, Grimes Golden, Delicious, Jonathan, and Stayman usually gave over 80 per cent clean trees, whether

TABLE 4.—A comparison of the percentage of clean trees obtained from string- and tape-wrapped wedge and whip grafts

Variety	1928										1929									
	String-wrap										String-wrap									
	Wedge					Whip					Wedge					Whip				
	No.	Clean	No.	Clean	In-crease	No.	Clean	No.	Clean	In-crease	No.	Clean	No.	Clean	In-crease	No.	Clean	No.	Clean	In-crease
Wealthy .....	530	74	348	55	19	Per cent	Per cent	Per cent	Per cent	Per cent	2091	73	1601	55	18	501	90	434	83	7
Yellow Transparent ..	509	72	124	68	14	cent	cent	cent	cent	cent	894	74	498	53	21					
Sweet Russet .....	1252	94	1362	66	28															
Whitney .....	362	91	348	67	24						1308	88	865	71	17					
N. W. Greening .....	640	75	857	62	13						831	89	658	82	7					
Jonathan .....	520	82	459	76	6															
Grimes Golden .....	227	91	101	85	6						715	88	278	85	3					
Delicious .....											380	94	175	93	1					
Duchess .....											532	76	581	54	22					
Total .....	4040	84	3599	66	18						6751	81	4656	65	16	501	90	434	83	7

TABLE 4.—(Continued)

Variety	1930									
	String-wrap					Tape-wrap				
	Wedge		Whip		In-crease	Wedge		Whip		In-crease
	No.	Clean	No.	Clean		No.	Clean	No.	Clean	
		<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>
Wealthy .....	958	82	1089	75	7	1140	94	1104	88	6
Yellow Transparent .....	695	77	662	75	2	673	91	688	87	4
Sweet Russet .....										
Whitney ..	196	96	190	81	15	196	99	157	92	7
N. W. Greening ..										
Jonathan ..										
Grimes Golden ..										
Delicious ..										
Duchess ..										
Total .	1849	80	1941	75	5	2009	93	1949	87	6

tape or string-wrapped wedge or tongue grafts were employed. In 1928, as shown in Table 4, the wedge graft increased the percentage of clean trees by 18 per cent and in 1929 by 16 per cent, while in 1930 a difference of only 5 per cent was found. When the tape wrapper was used, the wedge graft still showed 6 or 7 per cent increase in clean trees over the whip graft. It is to be noted that the percentage of clean trees in the whip grafts wrapped with string is higher in 1930 than in 1928 and 1929. This might be accounted for either by the fact that the nurserymen were exercising more care in the making of the grafts or by the dry season.

Several experiments were conducted in which the double-tongue graft also was used. Table 5 shows a summary of the results obtained by the

TABLE 5.—Results of the use of the double-tongue graft on the control of callus knot

Variety	Year	Wrapper	No. trees	Percentage clean trees
N. W. Greening	1928	Raffia	961	60.4
Wealthy	1928	"	900	42.5
"	1928	String	1210	64.8
"	1930	"	344	54.9
"	1930	Tape	322	85.4

use of this type of graft. In only one case did the double-tongue produce a higher percentage of clean trees than the whip graft. This was on Wealthy in 1928. The data show that at no time was there any question as to the superiority of the wedge graft for the control of callus knot when compared to the double-tongue graft. The use of nurseryman's tape as a wrapper

was also beneficial in the case of the double-tongue graft but not to such an extent as to give better results than the other 2 types of grafts.

Since there is a considerable difference between the growing season in Iowa and that of various other apple-growing sections, it is possible that this fact might influence the amount of callus knot found in the various localities. Table 6 shows the precipitation and temperature for certain

TABLE 6.—*A summary of certain meteorological data for certain localities in the States of Oklahoma, Kansas, Kentucky, Nebraska, and Iowa (10)*

State	Precipitation	Average temperature for 20 years		
	Annual	Mean annual	Normal	
			March	November
	<i>Inches</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>
Oklahoma ... ..	31.15	59.4	50.0	48.8
Kansas ..... .	37.65	55.3	44.5	44.1
Kentucky ..... .	43.3	55.0	43.7	44.8
Nebraska . . . . .	27.7	50.6	37.0	38.5
Iowa ..... . . . .	32.04	49.5	35.9	38.4

points that are nearest to the nursery sections in each of the 5 States. It is noted that the temperatures for March and November are higher in Oklahoma, Kansas, and Kentucky, which would give a longer period of growth in those States. There also is some difference in the annual precipitation of Kansas and Kentucky and Oklahoma, Nebraska, and Iowa. A summary of the data from all the apple trees examined in the 5 different States for the 3-year period is shown in table 7. This table shows that the smallest percentage (58 per cent) of clean trees produced from the wedge graft, was found in Oklahoma, while, in Kansas, 69 per cent clean trees were found. In Iowa 83 per cent was found and in Nebraska 86 per cent. From the trees examined, Kentucky showed the highest percentage of clean trees. However, when the grafts were wrapped with tape, as indicated by results from three States, these States still held their rank, while the percentage of clean trees increased by 10 per cent or more.

Although the percentage of clean trees produced from the different types of grafts is important, it also is advantageous to know where the callus knots were produced on the affected trees. This information would be of value in the making of grafts so that care could be taken to avoid those practices that are apt to cause callus knot. A summary of the location of callus knots found on the 3 types of grafts is shown in table 8. The whip



TABLE 7.—*Summary of three years' results, showing the effect of the geographical location on the percentage of overgrowths found at the union of piece-root-grafted apple trees*

Location	Totals									
	String-wrap					Tape wrap				
	Wedge		Whip		In-crease	Wedge		Tape		In-crease
	No.	Clean	No.	Clean		No.	Clean	No.	Clean	
		<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>
Oklahoma	739	58	1000	68	— 10					
Kentucky	682	96	783	94	2					
Kansas .....	1412	69	1488	61	8	997	87	961	81	6
Nebraska	9909	86	5505	78	8	725	97	697	93	4
Iowa ...	4247	83	4871	63	20	1776	96	871	90	6
Total .....	16,989	83	13,647	71	12	3498	93	2529	87	6

graft shows the highest percentage of callus knots on the scion lip and the side of the union. In the case of the double-tongue graft the callus knots at the scion lip have been reduced because the scion lip is inserted into a second cut in the stock. However, there are more cuts exposed on the side of the union and, as indicated in table 8, the majority of the callus knots occur at this point. The wedge graft shows a distribution of callus knots in 3 places, scion lip, side of the union, and exposed scion above the union. The callus knots produced at the scion lips may be due to 2 factors: firstly, the scion is too large for the stock as shown in figure 1, D, *d*, or, secondly, the

TABLE 8.—*A summary of the location of callus knots found on string-wrapped whip, wedge, and double-tongue grafts*

Type of graft	Knots examined	Location of callus knots				
		Scion lip	Stock lip	Side union	Exposed scion above union	Shoulder of stock
Whip .....	4775	<i>Per cent</i> 35.8 <sup>a</sup>	<i>Per cent</i> 10.8	<i>Per cent</i> 52.1	<i>Per cent</i> 1.3	<i>Per cent</i> 0.0
Double-tongue ...	1312	8.5	8.7	82.8	0.0	0.0
Wedge .....	2152	29.2	0.0	40.7	19.4	10.7

<sup>a</sup> Percentage of knots examined.

scion is broken over in cultivation, principally by the practice of "hooking" for the removal of weeds, so that the scion lip projects out beyond the stock. The callus knots produced at the side of the union might be remedied by a more even cut and a closer fitting of the cambium of scion and stock. The callus knots on the exposed scion above the union (Fig. 1, D, c) can be eliminated by inserting the scion into the stock until the cut surface is covered. All 3 types of grafts show about the same percentage of callus knots produced from the upper portion of the stock.

Some objections to the use of the wedge graft have been made because of the reported tendency (1) toward the development of sprouts from the graft shoulder. However, in all of the thousands of wedge-grafted trees observed, no such sprouts were found when the trees were 2 years old.

#### DISCUSSION

It is evident from the results presented that the wedge graft, whether wrapped with string or nurseryman's tape, gives a higher percentage of clean trees than the whip or double-tongue grafts. Tests of the breaking strength of the grafts show that when properly made and handled, the wedge graft is as strong as the whip or double-tongue grafts. Stand counts have shown that the difference in percentage of stand between the wedge and whip grafts is small, one type of graft showing perhaps 5 per cent better on one variety one year, and the next year, or in another locality, showing no difference, or the relationship may be reversed. Callus knots were found on the wedge graft in 3 locations: scion lip, side of the union, and exposed scion above the union. A remedy for these callus knots has been suggested. The use of nurseryman's tape largely prevents the formation of excess callus by covering the wounded tissues. This is indicated by the fact that an increase in clean trees of over 10 per cent is obtained in most cases. The evidence presented here justifies the recommendation of the wedge graft, wrapped with tape, for the control of overgrowths at the graft union.

It is noted that the percentage of overgrowths varies somewhat according to the variety. The wedge graft is especially recommended for the more readily callusing varieties, such as Wealthy, Yellow Transparent, and Duchess. Table 6 shows that the greatest increase in percentage of clean trees, due to the use of the wedge graft, was on those more readily callusing varieties, while on the more slowly callusing varieties such a marked increase was not present. However, it should be noted that such varieties as Jonathan, Delicious, and Grimes Golden showed rather high percentages of clean trees, even when the whip graft was used. An increase of about 5 per cent in clean trees was obtained by the use of the wedge graft on this group of varieties.

## - SUMMARY

A study of the mechanical strength of wedge, whip, and double-tongue grafts wrapped with string showed no significant differences. When the grafts were wrapped with nurseryman's tape, the strength was increased about 3 pounds regardless of the type of graft. A well-fitted graft is twice as strong as a poorly fitted one of the same size.

No marked difference was found between the stand obtained from wedge and whip grafts. From a limited number of data, the double-tongue graft showed a lower percentage of stand than the wedge and whip grafts. The kind of wrapper used, whether string or tape, did not have any significant effect upon the percentage of stand except in the case of the wedge and whip grafts during a dry season, when the tape wrapper showed a 10 per cent lower stand. Under the droughty conditions of the summer of 1930 the tape-wrapped wedge and whip grafts showed some increase in stand when scions of a caliper of over  $\frac{3}{16}$  inch were used. Other results with respect to caliper of scion showed no material differences in the percentage of stand obtained.

An increase in the percentage of clean trees was obtained on Wealthy, Yellow Transparent, Sweet Russet, Whitney, Northwestern Greening, Jonathan, Grimes Golden, Delicious, and Duchess varieties by using the wedge graft. Wealthy, Yellow Transparent, Whitney, and Duchess were varieties that showed the greatest increase from the use of the wedge graft. The least increase was obtained on Jonathan, Grimes Golden, and Delicious. The greatest increase obtained by the use of the wedge graft was 28 per cent on Sweet Russet in 1928.

The length of the growing season seemed to have an effect on the amount of callus knot, as a lower percentage of clean trees was obtained from grafts grown in Oklahoma and Kansas in comparison with the results obtained in Nebraska and Iowa.

The use of nurseryman's tape as a wrapper increases the percentage of clean trees about 10 per cent, whether wedge or whip grafted. The greatest increase due to wrapping with tape was 28 per cent on Wealthy whip grafts in 1929. The least increase was 3 per cent on Whitney wedge grafts in 1930.

The larger percentage of callus knots found on the wedge graft occurred on the scion lip, side of the union, and the exposed cut-surface of the scion above the union. Proper fitting and handling of the wedge graft would eliminate most of these knots.

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It was thought that possibly a rapid and abundant mycelial growth might be conducive to conidial production, so an attempt was made to find a medium upon which these species of *Cercospora* would grow readily. With this definite purpose, 30 kinds of media, which contained 40 different substances in varying quantities, were tried. It was convenient to classify these 30 kinds of media under four main groups: first, those containing cooked potato extract as the basic nutrient, plus additional salts and nutrients; second, those containing largely inorganic salts; third, host decoctions made by grinding the young succulent leaves of the respective host plants; and, fourth, those consisting wholly of sterilized host tissue.

The cultural characteristics of the 4 species of *Cercospora* used in the preliminary experiments, *C. beticola*, *C. davisii*, *C. dubia*, and *C. zebrina*, on the 4 main groups of media were as follows: In the first group, the growth was slow, aerial mycelium dirty gray, submerged mycelium olive green with numerous chlamydospore-like bodies; the aerial mycelium produced a gnarled, leathery mat usually forming oil droplets on the surface. In the second group, the growth was also slow, aerial mycelium gray, forming a gnarled, piled-up mat on the surface of the media with few chlamydospore-like bodies; oil droplets were produced over the surface of the mycelial mat. In the third group, the mycelial growth was dirty gray and much more rapid and abundant than in the other two, spreading over the entire surface of the media in about a week; very little submerged mycelium, and few chlamydospore-like bodies were produced. The nutrients in this group consisted largely of host decoction to which were added small amounts of dextrose and in some cases inorganic salts. Slowest growth was made on media in the fourth group; small amounts of light gray mycelium were produced, while in many instances there was cessation of growth in about 3 weeks.

It can be stated in general that those 4 species grew slowly, producing little mycelia on any of the media except numbers 14, 15 and 16. These media consisted of inorganic salts, juice from the beet plant and reduced agar base containing 1.2 per cent agar agar. Although these 4 species of *Cercospora* grew well on the media bearing the above numbers, no conidia were observed in any of the cultures at the end of a period of 2 to 3 weeks.

In view of the fact that no conidia were observed in the preliminary studies, new isolations were made of *C. beticola*, *C. davisii*, *C. dubia*, *C. physalidis* and *C. setariae*, in the fall of 1931 at Ames, and Kanawha, Iowa. D. H. Latham of Duke University very kindly supplied a culture of *C. cruenta* Sacc.

In the summer of 1932 4 additional species of *Cercospora* were collected and isolated, namely: *C. althaeina* Sacc., *C. avicularis* (Wint.) Hew., *C. muhlenbergiae* Atk., and *C. medicaginis* Ell. collected at Kanawha, Iowa.

*Cercospora mirabilis* Sharp and *C. moricola* Cooke were isolated from collections made by D. V. Layton at Conesville, Iowa.

*Conidial Production from Newly Made Conidial Isolations*<sup>3</sup>

Isolations were made from diseased leaves that had been placed in a moist chamber. After the leaves had remained in this environment from 15 to 20 hours, abundant production of conidia had formed in the necrotic spots. With the aid of a binocular microscope and a moistened, sterilized needle, there was little difficulty in isolating pure cultures of these fungi on potato-dextrose-agar slants. If a moistened, sterilized needle is brought just sufficiently near to the apical end of the conidia, the moisture on the tip of the needle will cause them to dislodge and adhere to the needle tip. These conidia, when transferred to agar slants, give a high percentage of pure cultures.

Certain species of *Cercospora*, as *C. dubia*, were difficult to isolate in pure culture, unless the above method was used. It appears that the conidia of this species were not so easily dislodged at the basal end as in the other species considered. However, if a leaf producing conidia remains in the moist chamber for about 15 to 20 hours, the conidia become easily dislodged and may be readily isolated in pure culture.

Within 36 to 72 hours after isolation small sparse mycelial colonies were produced on the surface of the nutrient-agar slants. Microscopic examinations showed (Table 1) that abundant conidial production had taken place in the following cultures: *Cercospora beticola*, *C. dubia*, *C. cruenta*, *C. davisii*, *C. physalidis*, and *C. setariae*.

Transfers were made from these isolations to potato-dextrose and sugar-beet-leaf-agar slants, and continued to sporulate when transferred at regular intervals to fresh agar slants. The method used in making these conidial transfers consists first of plunging a heated transfer needle into a sterile nutrient agar slant; then, by lightly scraping the moist, cooled needle over the surface of the culture, abundant conidia are caused to adhere to it. These conidia were transferred to the fresh nutrient-agar slant by lightly scraping the needle bearing the conidia over the entire surface of the medium to insure the development of a large colony.

The greatest number of conidia are produced by the conidiophores developed from the young mycelia arising from conidia. As the developing mycelia progressively covered the surface of the medium, fewer and fewer conidia were produced. There was greater vegetative growth and less sporulation in the case of *Cercospora beticola* on potato-dextrose agar, while the same organism on beet-leaf agar produced little vegetative

<sup>3</sup> By "newly-made isolations" is meant the isolation of conidia from diseased specimens collected from the living plant or from a recently collected specimen.

TABLE 1.—The sporulation of 6 species of *Cercospora* in pure culture

Cercospora species	Isolations		Pathogenicity	Cultural methods in maintaining sporulations			Interval of sporulation after transfer		Extent of sporulation
	Date	Host		Period of sporulation in months	Number of generations	Transfer intervals in days	Starting in hours	Stopping in days	
<i>C. beticola</i>	10/22/31	<i>Beta vulgaris</i>	+	3	20	3	40	8-12	Abundant
<i>C. cruenta</i>	1/10/32	<i>Vigna sinensis</i>	-	1.4	7	4	48	10-14	"
<i>C. davisii</i>	10/22/31	<i>Melilotus alba</i>	+	3	20	4	48	10-15	"
<i>C. dubia</i>	10/22/31	<i>Chenopodium album</i>	+	2	12	6	72	10-15	Numerous
<i>C. physalisii</i>	10/22/31	<i>Physalis</i> sp.	-	2	12	5	72	10-12	"
<i>C. setariae</i>	10/22/31	<i>Setaria glauca</i>	+	3	20	3	36	10-12	Abundant

growth and greater numbers of conidia for the first 4 or 5 days. However, the fungus seemed to grow freely thereafter, vegetatively, until the tenth day; then the growth was slow and was soon checked by dessication of the medium.

The amount of conidial production is affected by the kind of nutrient substratum, although the failure to culture these fungi on a specific medium did not prove to be the limiting factor in conidial production. *Cercospora beticola* fruited most abundantly when cultured on sugar-beet-leaf agar.<sup>4</sup> *C. cruenta*, *C. davisii*, *C. dubia*, *C. physalidis*, and *C. setariae* fruited abundantly on ordinary potato-dextrose agar.

When the sugar-beet-leaf medium was tubed and sterilized for 15 minutes at 12 pounds' pressure, it retained its leaf-green color. If the sterilization process is greatly prolonged, or at higher pressure, the medium becomes discolored and apparently is less suited to the growth of *Cercospora beticola* as shown by decreased conidial production.

A reduction in the amount of agar agar from the standard formula of 1.5 per cent to that of 1.2 per cent per liter solution was more conducive to conidial production. By using 1.2 per cent agar agar a medium is produced that is sufficiently firm to maintain the slants, but produces a comparatively soft substratum with more free water throughout, as well as on the surface of the nutrient substratum. These fungi grow more rapidly and sporulate more abundantly under such conditions.

As reported by Coons and Larmer (2), heavy mycelial growth was not followed by conidial production or especially conducive to it. This fact was verified in this laboratory; although in some cases a few conidia were produced on cultures where the mycelial growth was abundant, the most desirable type of medium for culturing these fungi was one on which very little aerial mycelium was produced during the first 5 or 6 days after conidial transfer. As shown in table 1, the minimum time for conidial production after conidial transfer varied with the species of *Cercospora*. The minimum time was 36 hours, the maximum, 72 hours, in case of *C. setariae* and *C. dubia*, respectively.

A medium suited to a particular species for optimum conidial production may not prove satisfactory in the case of other species of *Cercospora*. This is shown by *C. beticola*, which grew rapidly and fruited most abundantly on sugar-beet-leaf agar, while a 1.2 per cent potato-dextrose agar

<sup>4</sup> The sugar-beet-leaf agar used was a modification of "Coon's cheap synthetic medium" and consisted of 800 grams of freshly picked, young sugar-beet leaves, and 12 grams of agar agar per liter of solution. The sugar-beet leaves were finely ground to express the cell sap. This was then added to 1000 cc. of distilled water that contained 1.2 per cent melted agar agar and boiled 5 minutes, then strained through 2 thicknesses of cheesecloth, tubed, and autoclaved for 15 minutes at 12 pounds pressure.



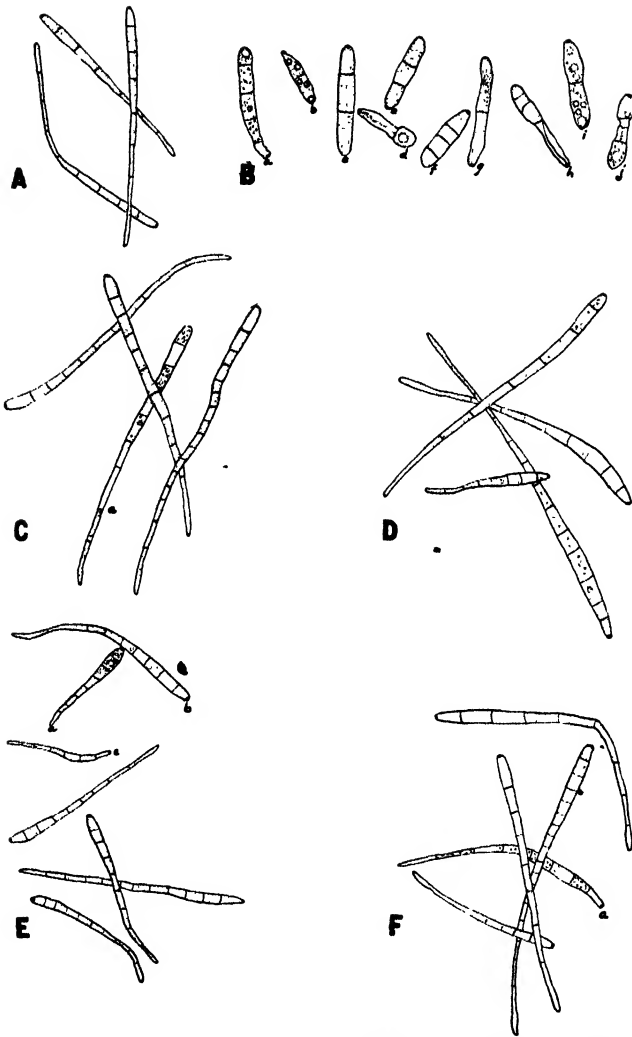


FIG. 1. Camera-lucida drawings of conidia from 6 species of *Cercospora* produced in pure culture.  $\times 880$ . A. *C. physalidis*. Normal conidia from 5-day-old culture. B. *C. dubia*. c, e, f, and g are normal conidia from 5-day-old culture. a, b, d, h, i, and j from 2- to 3-week-old cultures. These partially collapsed or disintegrated conidia are commonly found in 2- to 3-week-old cultures of the species of *Cercospora* studied. C. *C. cruenta*. Normal conidia from 5-day-old culture, except a, which shows empty cells within a conidium from 2-week-old cultures. D. *C. davisii*. Normal conidia from 5-day-old culture. E. *C. setariae*. Normal conidia from 3-day-old culture. a, b, and c from 2- to 3-week-old cultures. Large oil globular formations are taking place, with c showing one empty cell in the process of disintegration. F. *C. beticola*. Normal conidia of 5-day-old cultures except a, which shows partially disintegrated conidia from 2-week-old culture.

produced abundant conidia in the case of *C. cruenta*, *C. davisii*, *C. dubia*, *C. physalidis* and *C. setariae*. It is of interest to note that *C. setariae* fruited equally well on potato-dextrose and on sugar-beet-leaf agar.

Normal conidia were produced in pure culture by 6 species of *Cercospora*, as is shown in figure 1. The conidia were all hyaline and exhibited a wide range of measurements. Conidia of *C. beticola* ranged from 65 to 450  $\mu$ , *C. cruenta* 30 to 250  $\mu$ , *C. davisii* 50 to 203  $\mu$ , *C. dubia* 25 to 102  $\mu$ , *C. physalidis* 55 to 210  $\mu$ , *C. setariae* 40 to 175  $\mu$ . Figure 1 also shows that after these cultures were 2 or 3 weeks old, few normal conidia were found. Certain of these conidia apparently "break down" or become disintegrated. In the early stage of this disintegration the smaller oil globules, present in the older conidia, coalesce to form a few large globules. This is followed by a shrinking of the cell content within an individual cell of a conidium. As this process continues, the cell or cells of a conidium will become completely devoid of their cell contents. Whether a small portion of the cell wall disintegrates where the germ tube normally would develop or at some other point is not known. It is also possible that the cell walls are partially dissolved away by the "staling products" thought to be produced under pure-culture conditions. This is merely supposition, but the fact that the condition stated does occur may explain why other investigators have failed to observe conidial production in cultures that were several weeks old. In other cases conidial germination in culture was also observed.

Hopkins (6) and Schmidt (14) reported the finding of conidia in old cultures. This might be explained by a rapid drying out or desiccation of the media, in which case a complete disintegration and germination of all the conidia possibly would be prevented. Abundant infection was obtained in cases where the respective host plants were inoculated with a conidial suspension of conidia grown in pure culture. Complete host-inoculation studies were omitted, since host plants were unavailable.

During the summer of 1932 at Kanawha, Iowa, 6 additional species of *Cercospora* were collected and isolated, namely: *C. althaeina*, *C. avicularis*, *C. muhlenbergiae*, *C. moricola*, *C. mirabilis*, and *C. medicaginis*. These fungi were grown on potato-dextrose agar with the exceptions of *C. mirabilis* and *C. moricola*, which were grown on sugar-beet-leaf agar. The same technique as used as that followed in culturing the preceding species. The cultures were held at a temperature of approximately 25° C. over a period of 2 months and during this time 12 consecutive conidial transfers were made at 5-day intervals. Throughout the entire period abundant conidia were produced.

## MAINTAINING CULTURES IN A SPORULATING CONDITION

When it had been learned how to induce species of *Cercospora* to fruit from isolated conidia, the matter of maintaining these cultures in such a state became the consideration. In an attempt to determine the influence of interval of transfer on conidial production, a series of potato-dextrose agar and sugar-beet-leaf agar slants were planted with conidia, the latter being used for *C. beticola* only. These cultures were run in triplicate with 10 replications in each case. This series began with transfers from cultures 3 days old and continued with cultures 15 days old. Transfers made from 3- to 8-day-old cultures showed comparatively little difference in habit of growth and amount of sporulation. This may be explained by the fact that cultures at 8 days still retained an abundant supply of normal conidia. Although many conidia had germinated or disintegrated until only a few normal cells were present, these, when transferred to fresh slants, gave rise to new colonies. However, as the transfer intervals were lengthened there was macroscopic evidence that more vegetative growth was taking place than in cultures with shorter transfer intervals. Thus, by continuing this procedure, and by macroscopic as well as microscopic observations, the duration of conidial production (Table 1) was determined. During these 7 to 20 consecutive transfers sporulation was maintained, although at the end of this time there was some evidence of loss of vigor as compared with newly isolated cultures. In order to maintain some species of *Cercospora* in a sporulating condition it seems to be necessary to transfer spores rather than mycelium.

Subsequently, transfers were made from cultures varying in age from 1 to 5 months. In most cases there were a few conidia, but they were less abundant than in cultures that had been transferred at regular intervals of 5 to 6 days. The cultures also grew more slowly and produced many sterile hyphae. In general, the transfer of mycelia yields only sterile hyphae. Coons and Larmer (2) also observed the tendency of cultures to lose their power of fruiting when grown in artificial culture for long periods of time.

## SUMMARY

The following 12 species of *Cercospora* were studied: *C. althaeina*, *C. avicularis*, *C. beticola*, *C. cruenta*, *C. davisii*, *C. dubia*, *C. muhlenbergiae*, *C. moricola*, *C. mirabilis*, *C. medicaginis*, *C. physalidis*, and *C. setariae*.

In preliminary experiments, using transfers from stock cultures of *C. beticola*, *C. dubia*, *C. davisii*, and *C. zebrina*, to 30 different kinds of media, no conidial production was observed.

Transfers from sporulating cultures yielded abundant spores, while transfers of mycelium gave largely sterile hyphae.

New isolations yielded abundant conidia in pure culture under favorable conditions in 36 to 72 hours.

Through the use of definite transfer intervals ranging from 4 to 6 days, conidia producing cultures were maintained from 5 weeks to 3 months.

Certain of these species of *Cercospora* demonstrated some specificity of medium, as determined by the amount of sporulation.

BOTANY AND PLANT PATHOLOGY SECTION,  
AMES, IOWA.

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abundant conidial production with *C. beticola*, *C. davisii* Ell. & Ev., *C. physalidis* Ell., *C. setariae* Atk., and *C. zebrina* Pass.

Schmidt (14) reports that *Cercospora beticola* seldom produces conidia in pure culture, and when it does, only sparingly. He obtained best results upon placing the cultures at temperatures of 25° C. for a short period and then at a cooler temperature of 15° C. He found one culture, among many, that had been held under ordinary summer temperatures for several months, that contained numerous conidia. He was unable to offer any explanation for this particular case. Stolze (17) also stated that he was unable to observe sporulation while studying *C. beticola* in pure culture.

Jenkins (7) reported that typical conidia of *Cercospora cerasella* have been produced in pure cultures from conidia and ascospores from this fungus. However, he states further that when conidia were planted on such media as potato-dextrose, corn-meal agar, bean and dextrose agar, synthetic nutrient agar, or cherry-leaf-decoction agar to which were added fragments of diseased leaves as an additional source of nutrients, no fruiting was observed, although the fungus made a good vegetative growth. If, however, tubes of these media on which the organism had been growing for a time were again sterilized and planted with conidia, conidia were produced. Welles (18) dealing with several Philippine species of *Cercospora* obtained conidia in a few days in pure culture. However, Welles (19) in earlier studies with *C. melongenae* Welles, in which he kept certain cultures under observation for 5 weeks observed no fruiting. Singh (15) was successful in obtaining conidia in pure culture with *C. patouillardii* Sacc., *C. leucosticta* Ell. and a *Cercospora* sp., but failed to obtain conidia in the case of a fourth species, *C. feuilleaubeisii* Sacc.

#### PRELIMINARY EXPERIMENTS AND SOURCES OF CULTURES

The species of *Cercospora* used in the preliminary experiments were *C. beticola*, *C. davisii*, *C. dubia* (Riess) Wint., and *C. zebrina*. These were collected by S. M. Dietz and E. F. Vestal in the sugar-beet growing districts of northern Iowa. The isolations were made in the Botany and Plant Pathology laboratories at Ames, in the fall of 1930. These cultures were grown and maintained on potato-dextrose agar slants by E. F. Vestal until February 1, 1931. At that time transfers were obtained from each species for use in the following investigations.

In the preliminary trials the transfers were held under ordinary laboratory conditions from February, 1931, to August, 1931. During this time 30 consecutive mycelial transfers were made on potato-dextrose and sugar-beet-leaf agar slants. Microscopic examinations of the cultures were made in approximately 2 weeks from the date of transfer, when the fungus had produced a mycelial mat about 2 to 3 centimeters in diameter, but no conidia were observed in any of the cultures.

# CONIDIAL PRODUCTION IN SPECIES OF *CERCOSPORA* IN PURE CULTURE<sup>1</sup>

CLATUS M. NAGEL<sup>2</sup>

(Accepted for publication December 29, 1933)

Considerable difficulty was encountered in obtaining conidial production in pure culture in connection with a study of the host range of *Cercospora beticola* Sacc. The lack of abundant conidial production in pure culture with which to perform cross inoculations made it necessary to develop a method for obtaining abundant sporulation. Such a method has been found for the species of *Cercospora* studied. The effect of different media and a procedure for maintaining cultures in a sporulating condition are described in this paper.

Those who have investigated this problem present conflicting evidence regarding conidial production of not only *Cercospora beticola* but also other species of *Cercospora*. Duggar (3), working with *C. apii* Fres., in 1897, obtained conidia in pure culture when he grew the fungus on celery petioles and bean stems. The same investigator (4), however, in 1899, failed to obtain conidia in culture with *C. beticola*. He maintained cultures in the laboratory under various conditions over a period of 2 years, and reports that no type of fruiting was observed during his investigations. Klotz (9) reported conidial production with *C. apii* when he grew the fungus on celery refuse. Solheim (16) studied several species and was successful in obtaining conidia with only *C. ampelopsidis* Peck and *C. illinoensis* Barth.

Garman (5) reports that *Cercospora brunckii* Ell. & Galw. grew well on several media, but that it did not readily produce conidia. Jones and Pomeroy (8), McKay and Pool (10), Rand (13), Welles (19), and Wolf (20) reported similar difficulties, though other investigators were more successful. Aderhold (1) obtained conidia with *C. cerasella* Sacc., Coons and Larmer (2), working with *C. beticola*, observed conidia, produced somewhat sparingly over the entire mycelial mat, but in greater numbers at the borders of the colonies. They grew the cultures on a sugar-beet-leaf medium.

Hopkins (6) attributed his success in conidial production to a partial drying out of the potato-dextrose agar in plate cultures of *Cercospora medicaginis* Ell. & Ev. Nagel and Dietz (12) and Nagel (11) reported

<sup>1</sup> Journal paper No. J138 of the Iowa Agricultural Experiment Station, Ames, Iowa. Botany and Plant Pathology Section Project No. 76.

<sup>2</sup> The writer wishes to express his appreciation for the helpful advice and criticism given by Dr. S. M. Dietz during the course of the investigational work and the preparation of this manuscript.

# APPLICATION OF STREAM DOUBLE REFRACTION IN THE IDENTIFICATION OF STREAK DISEASES OF TOMATO<sup>1</sup>

WILLIAM N. TAKAHASHI AND T. E. RAWLINS

(Accepted for publication November 20, 1933)

Several workers have demonstrated that necrosis of tissues in stems, leaves, and fruits of the tomato plant may be caused by infection with different viruses or combinations of viruses. Among the viroses showing this symptom are those commonly called the "streak" diseases.

We have been concerned with two of these diseases; one, which is well known and that several workers have called "combination streak," is caused by the potato latent virus when combined with tobacco-mosaic virus; the other, for which Shapovalov (2) proposed the name "dieback streak," is caused by a virus, or combination of viruses, which has not been identified. The combination streak was first described by Johnson (1) and the dieback streak by Shapovalov (2). As is evident from the descriptions given by these workers, the symptoms of the 2 diseases are somewhat similar and in certain cases it is difficult to distinguish them.

We have recently studied the stream double refraction exhibited by juice from tomato plants manifesting these diseases with the hope that this technic might be applicable in differentiating them.

Some of the specimens of dieback streak were taken from plants grown in a greenhouse at Riverside, California, and artificially inoculated by M. Shapovalov with this streak (all "B" specimens in table 1); others were naturally infected plants collected by Shapovalov in southern California (all "Q" specimens in table 1) and by M. W. Gardner and O. C. Whipple in central California (all "G" specimens in table 1). The combination streak used was always produced in the greenhouse at Berkeley by inoculating plants with a mixture of the potato latent virus and tobacco-mosaic virus.

Since previous experiments (4) have indicated that the method of juice extraction has a marked influence on stream double refraction 3 methods of extraction were used throughout these experiments. In the tables MC indicates that the tomato tops were macerated in a mortar and the juice was pressed out and centrifuged; MCFC indicates that juice obtained as above was frozen overnight at 0° F. and then thawed and recentrifuged; MFC indicates that the macerated tissue was frozen overnight, thawed, pressed

<sup>1</sup> Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

TABLE 1.—Summary of stream double-refraction experiments in the identification of streak diseases of tomato

Disease	Plant number	Date	Critical dilution			Number of Nicotiana glutinosa leaves inoculated	Average number of lesions per leaf	Locality where plants were collected
			MC	MCFC	MFC			
Combination streak	1	5-25-33	1-256	.....	1-341	.....	.....	Greenhouse (inoculated)
"	2	"	1-256	.....	1-256	.....	.....	"
"	3	"	1-341	.....	1-512	.....	.....	"
"	4	"	1-256	.....	1-341	.....	.....	"
"	5	"	1-512	.....	1-768	.....	.....	"
"	6	8-19-33	1-512	.....	1-1024	.....	.....	"
"	7	"	1-256	.....	1-768	.....	.....	"
"	8	"	1-512	.....	1-341	.....	.....	"
Tobacco mosaic	1	8-19-33	1-512	.....	1-768	.....	.....	"
"	2	"	1-128	.....	1-196	.....	.....	"
"	3	"	1-256	.....	1-341	.....	.....	"
Dieback streak	B-1a	.....	1-512	.....	1-768	.....	.....	"
"	B-2a	.....	1-16	.....	Slight	.....	.....	Southern California
"	B-3	.....	1-16	.....	"	.....	.....	"
"	B-4	.....	1-128	.....	1-8	.....	.....	"
"	B-5	.....	1-16	.....	1-4	.....	.....	"
"	B-6	5-29-33	1-47	.....	Slight	.....	.....	"
"	B-7	"	1-16	.....	0	.....	.....	"
"	B-1b	"	1-32	.....	1-2	.....	.....	"
"	B-2b	"	1-24	.....	1-3	.....	.....	"
"	Q1	8-3-33	1-4	.....	Slight	.....	.....	"
"	Q2	"	1-16	.....	Slight	.....	.....	"
"	Q3	"	1-16	.....	1-4	.....	.....	"
"	Q4	8-9-33	1-8	.....	1-6	.....	.....	"
"	Q5	"	1-8	.....	Slight	.....	.....	"
"	Q6	"	1-8	.....	1-2	.....	.....	"
"	Q7	"	1-6	.....	1-3	.....	.....	"
"	Q8	"	1-12	.....	Slight	.....	.....	"
"	Q9	"	1-12	.....	1-3	.....	.....	"
"	Q10	"	1-8	.....	0	.....	.....	"
"	Q12	"	1-16	.....	1-6	.....	.....	"



TABLE 1—(Continued)

Disease	Plant number	Date	Critical dilution			Number of Nicotiana glutinosa leaves inoculated	Average number of lesions per leaf	Locality where plants were collected
			MC	MCFC	MFC			
Dieback streak	Q13	8-9-33	1-6	Slight	Slight	5	0	Southern California
"	Q14	"	1-8	"	1-6	5	0	"
"	Q15	"	1-8	1-4	Slight	5	0	"
"	G1	6-1-33	1-8	Slight	Slight	..	..	Clayton
"	G2	"	1-12	1-3	1-3	..	..	Concord
"	G5	6-6-33	1-12	1-4	1-4	10	0	Merced
"	G6	6-5-33	1-6	Slight	Slight	10	0	Concord
"	G7	6-17-33	1-16	1-6	1-4	10	0	"
"	G8	"	1-12	1-4	1-4	10	0	"
"	G9	6-16-33	1-12	1-4	1-4	10	0	San Jose
"	G10	6-14-33	1-16	1-8	1-8	10	0	Concord
"	G12	6-16-33	1-12	1-8	1-8	10	0	San Jose
Normal plant	1	5-25-33	1-32	1-6	Slight	..	..	Greenhouse
"	2	"	1-6	1-2	0	..	..	"
"	3	"	1-24	1-6	Slight	..	..	"
"	4	"	1-32	1-3	0	..	..	"
"	5	"	1-32	1-4	0	..	..	"
"	6	"	1-47	1-4	0	..	..	"

and the juice centrifuged. The stream double-refraction technic described in an earlier paper (3) was used. In each test the juice was diluted with distilled water until stream double refraction was no longer detectable. This dilution we have called the critical dilution and have indicated it as 1-16 etc. in the tables. "Slight" and "0" indicate that even the non-diluted juice showed very slight or no double refraction, respectively.

The juice prepared by maceration of tissues, followed by freezing overnight, pressing, and centrifuging, (MFC) was diluted with an equal volume of water and was brushed on the leaves of *Nicotiana glutinosa* plants in order to determine whether tobacco-mosaic virus was present in the plants under test. The average number of local lesions produced per leaf is shown in table 1.

As is shown in table 1, juice from plants having dieback streak exhibited stream double refraction indistinguishable from that shown by juice from healthy plants. In this disease the critical dilution was always low and under MCFC and MFC was lower than under MC. Inasmuch as the juice from these plants produced no local lesions on *Nicotiana glutinosa*, the absence of tobacco-mosaic virus was proved. (It is possible that the virus or viruses causing dieback streak might have produced symptoms on *N. glutinosa* had the leaves been inoculated immediately after the juice was extracted.)

Plants having combination streak regularly exhibited a very high critical dilution, which was usually higher under MCFC and MFC than under MC. The stream double refraction exhibited by combination streak was indistinguishable from that shown by plants infected with tobacco-mosaic virus alone.

The above results demonstrate that the stream double-refraction technic may be used to distinguish plants having dieback streak from those infected with combination streak.

#### SUMMARY

Juice from streaked tomato plants infected with a combination of tobacco-mosaic and potato latent viruses exhibits a stream double refraction indistinguishable from that exhibited by tomato plants infected with tobacco-mosaic virus alone.

Juice from tomato plants infected with dieback streak exhibits a stream double-refraction behavior, which is indistinguishable from that shown by normal plants. This technic may therefore be used to distinguish diseased plants infected with combination streak from those infected with dieback streak.

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## PHYTOPATHOLOGICAL NOTES

*Cytological Changes in the Callus of the Graft Union in Connection with Curly Top in Tomatoes.*—It was reported by Shapovalov<sup>1</sup> that when an approach graft is made to unite a healthy tomato plant with a virus-affected one, as in the case of the curly-top infection, transmission of the virus occurs in a majority of cases. However, there is always a small percentage of grafts in which virus transmission does not result. A microscopic study of the tissues in the graft region reveals peculiar changes in the cell structure.

Callus tissues, including the graft union, were killed in Némec killing fluid, sectioned 5  $\mu$  thick, stained with acid fuchsin, and counterstained with light green. In these sections the surface of the union was made apparent,



FIG. 1. Cellular structure through a graft union of tomatoes infected with curly top. B. Bridging over of proliferated cells. J. Cells that have reverted to meristematic condition. G. Degenerated cells at the surface of contact. M. Mitochondria. V. Vacuoles. A. Amyloplasts. N. Nucleus.

<sup>1</sup>Shapovalov, M. Graft transmission of curly top in tomatoes (tomato yellows). *Phytopath.* 21: 998-999. 1931.

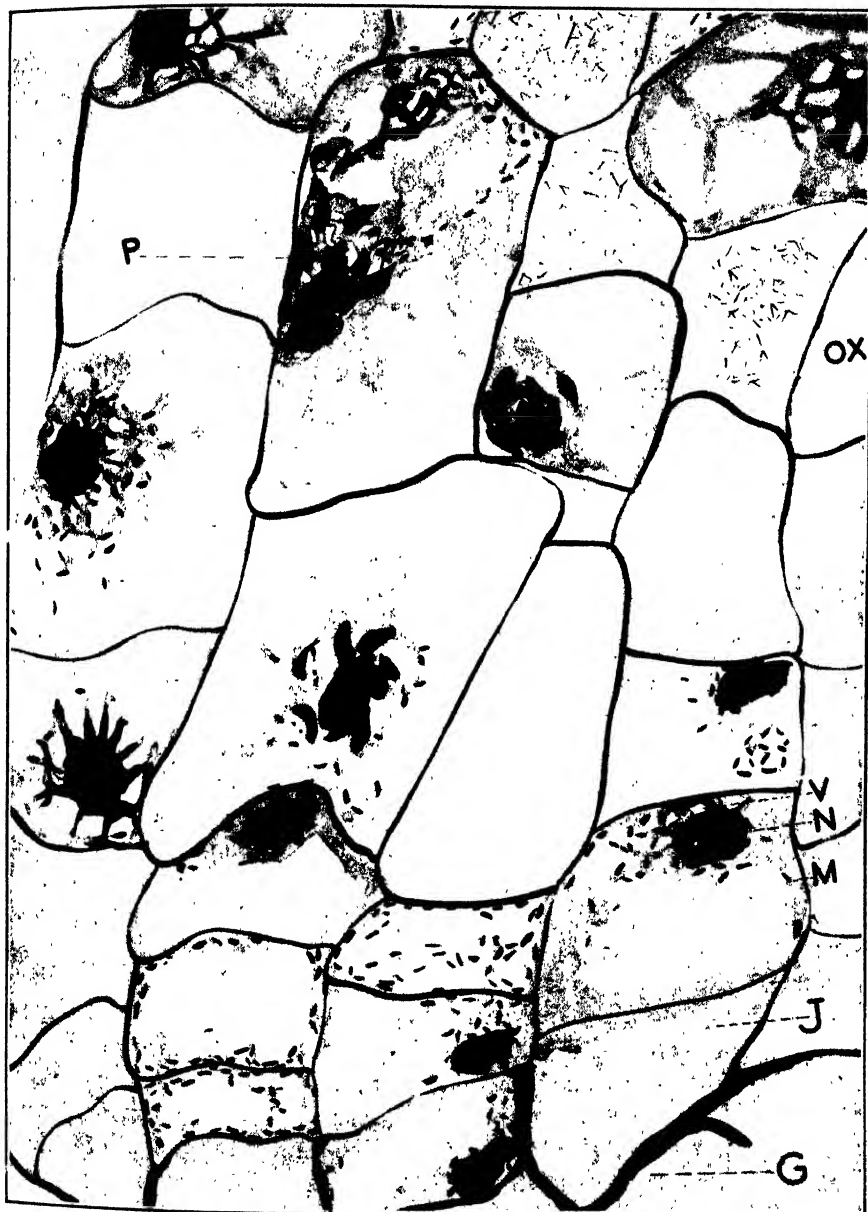


FIG. 2. Formation of calcium oxalate crystals near the contact surfaces of the graft union in a curly-top-infected tomato. OX. Crystals of calcium oxalate. P. Cells showing polarization, with amyloplastids grouped around the nucleus and numerous mitochondria grouped at the farther end of the cell. V. Vacuoles. N. Nucleus. M. Mitochondria. J. Meristematic condition of the cells, up and left. G. Degenerated cells at the graft union.

through united calluses, by a row of cells showing "gummy" (probably pectic) degeneration (Figs. 1 and 2, G). On each side of this row, cells reverted to the meristematic condition (Figs. 1 and 2, J). They contain no plastids, but only mitochondria (Figs. 1 and 2, M), and their cytoplasm is honeycombed with numerous small vacuoles (Figs. 1 and 2, V). Through a hyperplastic proliferation of these cells living tissues bridge across the row of degenerated cells, thus bringing about a contact between the living cells of the two plants (Fig. 1, B).

It may be readily seen, therefore, that after grafting, the virus can pass from one plant to another, although this movement is not so readily accomplished as in the tissues of the same plant, because of a barrier of degenerated cells.

Kostoff,<sup>2</sup> in grafting one species of *Nicotiana* to another, obtained precipitation phenomena in the expressed sap of the grafted plants, which he postulated to result from the formation of precipitins, as a response to grafting. In terms of antibody formation, he discussed his results as being of general significance in immunology.

Quite recently, Whitaker and Chester<sup>3</sup> gave a simple biochemical explanation of Kostoff's precipitation phenomenon. They pointed out that species of *Nicotiana* may differ as to the relative concentration of calcium and oxalic acid ions in their tissues. When the juices of 2 different species are mixed, as after grafting, calcium oxalate may be precipitated. Chemical tests in the laboratory proved that calcium oxalate is involved in this precipitation but no microchemical observation was made to localize the calcium oxalate in the tissues, *in vivo*.

It, therefore, seems of interest to note in this connection that large amounts of calcium oxalate were actually precipitated in some cells of the callus, a few layers away from the contact surfaces of grafted tomatoes (Fig. 2, OX).

Thus, the following principal changes were observed in the callus tissues formed at the graft union between an apparently healthy and a curly-top-infected tomato: (1) Death and a gummy degeneration of many of the cells adjacent to the contact surfaces of 2 grafted plants; (2) reversion to the meristematic condition of the underlying cells, and a bridging-over process by hyperplastic cells; and (3) a very abundant production of calcium oxalate in some cells, deeply situated in the callus.—J. DUFRÉNOY, Directeur Station de Pathologie Végétale du Sud-Ouest, Bordeaux, France, and MICHAEL SHAPOVALOV, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

<sup>2</sup> Kostoff, Dontcho. Acquired Immunity in Plants. *Genetics* 14: 37-77. 1929.

<sup>3</sup> Whitaker, T. W. and K. S. Chester. Studies on the precipitin reactions in plants. *Amer. Jour. Bot.* 20: 297-308. 1933.

*A Fungus Gall on Viburnum Mistaken for Crown Gall.*—Several years ago galls on *Viburnum opulus* L., the European cranberry bush, were received from an entomologist of New York State. He could find no insect, nor traces of any, to which responsibility for the trouble could be attached and thought it might be a case of crown gall. The shrubs affected were in a public park; the galls were spreading to other plants of the same species and some of the original infected ones had dead or dying branches. A few to many galls occurred on the stems and were  $\frac{1}{4}$  in. to 1 in. or more in diameter (Fig. 1). They occurred at and just above and below the nodes and usually encircled the stem. The outgrowths consisted of root primordia; and, as this condition may occur in crown gall, work was undertaken to establish the presence of *Bacterium tumefaciens*. That organism could not be isolated from either of two lots of material. A fungus, however, was found present in several galls of both lots. This was isolated and inoculated into young *Viburnum opulus* plants the following spring, while the plants were growing well. No infection came from inoculations with the fungus, so the cause had to be sought elsewhere. Requests were made to the superintendent of the park where the disease occurred to send specimens at various times in the summer when the galls were small or just starting to form. This he did, and on the young galls that arrived in midsummer tiny flies and eggs of mites were found. The former were identified as *Cecidomyia* sp., and at the time were thought to be responsible for the galls.

An interval of 10 years elapsed, during which the writer received galls on *Viburnum opulus* at various times. These were considered to be *Cecidomyia* galls. In the summer of 1933, however, the specimens she received in various stages of development were studied by staff members of the Bureau of Entomology who could find no insect responsible for the galls. So, in search of a pathogen, isolations were made from the 1933 material and the same fungus that was isolated in 1923 was obtained. Inoculations by means of needle pricks were made into *Viburnum opulus* and *V. tomentosum* Thunb. in the spring and summer of 1934. This time galls were produced on *V. tomentosum* in less than 2 months, but up to the present time, July 20, none has developed on *V. opulus*, the native host. The galls produced are of the nodular type of the original galls. The stems punctured with a sterile needle for controls showed no outgrowths.

As the fungus enters through a wound, it seems probable that a mite or aphid may be associated with the parasite in the production of galls.

The fungus responsible for the disease is a *Phomopsis*. It is being studied to learn its relationship to the *Phomopsis* species known to be pathogenic.—NELLIE A. BROWN, Bureau of Plant Industry, Washington, D. C.



FIG. 1. Galls on *Viburnum opulus* from which *Phomopsis* was isolated



*White Pycnia and Aecia of Puccinia Graminis.*<sup>1</sup>—In the routine testing of various species, varieties, and hybrids of *Berberis* for susceptibility to stem rust (*Puccinia graminis* Pers.), seedlings of *B. vulgaris* L. were inoculated in successive series with teliospores of *P. graminis agrostidis* Eriks. that had been stored at 0–2° C. for more than a year. Light to moderate infection resulted. Among the usual yellow pycnia there appeared a number of white pycnia. The pycniospores from both were hyaline, but the nectar of the white pycnia was colorless instead of the normal yellow. The difference in color, therefore, was in the nectar rather than in the spores.

Nectar was transferred from the white pycnia to white and to yellow pycnia. No aecia developed under the white pycnia treated with white nectar, but yellow aecia formed under some of the white pycnia treated with yellow nectar. Seedlings of *Agrostis alba* L. and of Manchuria barley were inoculated with these aeciospores, but no uredia developed.

A second set of inoculations was made on young barberry seedlings with teliospores of the same collection and a number of white pycnia again appeared. These infected barberries were placed under bell jars as soon as the infection became evident and were kept covered to exclude possible contamination. Ten days later nectar was transferred from white pycnia to white pycnia, from white pycnia to yellow pycnia, and from yellow pycnia to white pycnia. In a few cases aecia developed under the white pycnia treated with nectar from other white pycnia. These aecia were white, while those that developed under yellow pycnia treated with white nectar were yellow.

The number of “crosses” attempted is small, but the results indicate that white pycnia treated with white nectar form aecia about as readily as the normal yellow ones. Of the white × white crosses, 3 out of 7 resulted in the production of aecia. As a result of 23 crosses between white and yellow pycnia, 5 aecia appeared. No aecia formed under the one white pycnium treated with yellow nectar. As previously stated, the aeciospores formed under white pycnia to which white nectar had been transferred were white, while those formed under yellow pycnia to which white nectar had been transferred were yellow, as were the naturally formed aecia under the compound yellow pycnia. The length, size, rate of growth, etc., of the yellow aecia and the white aecia were similar under the conditions in the bell jars where the “crossing” was done and where the aecia formed.

<sup>1</sup> Cooperative investigation between the Minnesota Agricultural Experiment Station and the Project of Barberry Eradication, Division of Plant Disease Eradication, Bureau of Entomology and Plant Quarantine, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The investigation was undertaken before the Project of Barberry Eradication was separated from the Bureau of Plant Industry.

These white aecia were similar in size to and but slightly different in shape from the normal yellow aecia. The spores were hyaline and the intercalary cells in the chains were more conspicuous than those in the yellow chains. The aeciospores of both germinated normally in barley extract, and the germ tubes from the hyaline spores were hyaline.

A record was made of the numbers of white and yellow pycnia obtained from 2 sets of inoculations of successive series of *Berberis vulgaris* seedlings. In the first set there were, out of a total of 74, 21 white pycnia, constituting 28.38 per cent of the total number. In the second set of 102 there were 16 white pycnia, or 15.68 per cent. In the 2 sets the total number of pycnia was 139 yellow and 37 white, the white pycnia constituting 21.02 per cent of the total number in the 2 sets of inoculations.

The white pycnia were not distributed at random over the leaves of the infected barberries but were found more commonly on the leaves on one side of the plant. Also, the proportion of white to yellow pycnia on the same leaf was much higher on the one side of the barberry plant than on the other, and in a few cases white pycnia only were observed on leaves of the one side of the plant, while on the other side all were yellow. Thus, there were indications that the sporidia giving rise to white pycnia were produced on certain stems rather than at random throughout the collection of telial material, as the inoculations were made by suspending telial material above the barberry plants.—RALPH U. COTTER, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

*Mercury Ammonium Silicate as a Gladiolus Corm Treatment.*<sup>1</sup>—The effectiveness of calomel in the control of gladiolus scab has been proved.<sup>2,3</sup> Important commercial growers of gladiolus corms have reported its cost excessive when used at a concentration of 1 pound to 10 quarts of water, as recommended. In dipping corms in such a suspension it also was noted that the coverage of the corms was not uniform. In order to obtain a more even coverage of a toxic material, the possibilities of a mercury-ammonium silicate gel prepared by the action of a solution of mercuric bichloride on a mixture of ammonium hydroxide and sodium silicate were investigated.

Two formulae were employed in making the mercury silicate gels that differed only in solubility. The results were more satisfactory with the less soluble material. The coverage on the corms with this mercury gel was very uniform and complete, particularly when small amounts of a spreader were added.

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Pathology.

<sup>2</sup> Tilford, P. E. Corm treatment for gladiolus and calla lily. (Abst.) *Phytopath.* 21: 121. 1931.

<sup>3</sup> Miles, L. E. Control of gladiolus scab. *Phytopath.* 23: 802-813. 1933.

Seven commercial varieties of gladiolus have been used in these tests with a total of 34 replications. Tests have been conducted also on gladiolus corms infected with *Penicillium gladioli*.

In all cases the mercury-ammonium silicate dip was compared with the standard calomel dip and a 7-hour soak in mercuric bichloride 1-1000.

TABLE 1.—Summary of experiments in control of gladiolus scab

Treatment	Clean corms	Corms with			Mummies
		1-4 lesions per corm	5-10 lesions per corm	over 10 lesions per corm	
		1932			
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Check	23.4	39.8	22.3	12.7	1.8
HgCl <sub>2</sub>	49.7	36.5	7.3	2.6	3.9
Calomel	75.5	21.0	0.7	....	2.8
M. A. S. <sup>a</sup> .....	83.8	14.1		0.2	1.9
M. A. S. $\frac{1}{2}$ Hg. conc.	61.9	30.8	3.5		3.8
M. A. S. $\frac{1}{4}$ " "	52.4	31.4	9.6	4.8	1.8
		1933			
Check	17.3	42.6	19.9	13.0	7.2
HgCl <sub>2</sub>	57.2	38.0	4.5	....	0.3
Calomel	87.4	12.0			0.6
M. A. S. <sup>a</sup> .....	90.7	9.3		"	"
M. A. S. $\frac{1}{2}$ Hg. conc.	83.1	16.4	0.4		0.1
M. A. S. $\frac{1}{4}$ " "	69.1	30.2	0.6	0.1	...

<sup>a</sup> Mercury ammonium silicate with mercury concentration equal to standard calomel dip.

From an examination of the data presented in the table, it is evident that the less soluble compound used in 1933 gave better protection against scab infection than the more soluble compound used in 1932. In both cases, where the mercury silicate was used at mercury concentrations equal to the standard calomel dip, control was essentially the same. When used at one-half the mercury concentration of calomel, the effectiveness was markedly reduced in 1932 but only slightly so in 1933, when the less soluble gel was used.

The experiments with *Penicillium gladioli* in 1931, 1932, and 1933 were conducted on Gen. de Wett, a white-flesh, susceptible variety. Calomel in 1931 increased the percentage clean corms from 17.2 in the check to 22.0 in the dipped lot. Mercury-ammonium silicate in 1932 increased the percentage clean corms from 18.9 in the check to 49.7, while in 1933, the percentage clean corms was increased from 43.4 per cent in the check to 69.7

per cent in the treated lot.—RICHARD P. WHITE, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

*The Effect of Mosaic on Bloom Production of the Talisman Rose.*<sup>1</sup>—Observations in commercial rose houses indicated that mosaic infection on the Talisman variety did not markedly interfere with growth or bloom production. This was so strikingly unlike the reaction of the susceptible Madame Butterfly, which had been observed to produce less than 25 per cent of normal blooms, that the matter was thought worthy of investigating.

In April, 1932, the writer was called upon to rogue a large shipment of potted Talisman plants received from Oregon as dormant buds. When potted and grown for benching, they showed very vigorous growth. By the time they were ready for the bench, however, several had developed very marked and characteristic symptoms of mosaic. Thirty-six such plants were selected for planting in a small experimental greenhouse and 36 plants of the same lot, but showing no symptoms after a most careful examination, were selected for comparison purposes.

During the first 3 months of growth in the bed, 8 of the plants selected as healthy developed mosaic symptoms. During the following 21 months no additional mosaic plants were noted. This indicates that, at the time of benching, the most thorough examination of the growing plants is not adequate to effect a complete elimination of infected plants.

These plants, 28 healthy and 44 mosaic-infected, have been grown for 2 successive years in ground beds. The number of blooms cut, the length of stem from the base of the bud to the cut so made as to leave 2 leaves on the remaining stub, and the number of imperfect blooms have been recorded. During the second year of their growth notes also were taken on foliage symptoms of mosaic, since foliage showing severe symptoms would render that cut unsalable. A certain percentage of cuts showing slight symptoms on the foliage could probably be marketed.

A summation of the data for 2 production years showed that mosaic-free Talisman plants, under the conditions of this experiment, produced annually a total of 31.98 blooms per plant, with an average stem length of 10.77 inches. The mosaic-infected plants produced 34.78 blooms per plant with an average stem length of 10.55 inches. The increased number of blooms per plant on the latter is due to a tendency of mosaic-infected plants to lose the normal dominance of the terminal bud.

The percentage of imperfect blooms cut from the healthy and diseased plants was 2.90 and 7.55, respectively. During the second year, the cuts from the mosaic-infected plants showed 10.7 per cent with severe foliage

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Pathology.

symptoms and 7.5 per cent with slight foliage symptoms. The former were considered unsalable.

Assuming that the same percentage of cuts during the first year showed foliage symptoms rendering them unsalable, the production for the 2 years can be expressed on a basis of salable blooms per plant per year. On this basis the mosaic-free plants produced annually 31.05 blooms per plant and the mosaic-infected plants produced 29.07 blooms per plant.

These data indicate the relatively slight influence from a practical viewpoint that mosaic-infection has on the Talisman variety.—R. P. WHITE, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

*Increase of Tobacco-Mosaic Virus in the Absence of Chlorophyll and Light.*—In normal green leaves of certain varieties of the pepper, *Capsicum frutescens* L., infection with tobacco-mosaic virus is accompanied by production of necrotic local lesions. In variegated leaves of the same plants, white and apparently chlorophyll-free areas respond to inoculation by the production of similar lesions. Since the appearance of such lesions is regularly accompanied by and presumably dependent on virus increase, it seems to be indicated that multiplication of tobacco-mosaic virus is not dependent upon presence of chlorophyll.

This conclusion is supported by the results of a hitherto unpublished study of rate of increase of tobacco-mosaic virus in Green Mountain potato stems. Tubers were kept in pots of moist soil in a windowless cellar room, guarded by a double door against light from outdoors. A screen consisting of several layers of black cloth was placed around the pots to prevent accidental exposure to artificial illumination. When etiolated stems, with rudimentary leaves, had reached a length of a foot or more, pieces of stem 2 to 3 inches long were cut, inoculated with tobacco-mosaic virus by means of pin punctures, and inserted in previously sterilized test tubes. It is believed that these stems were free of chlorophyll. The work of cutting and inoculating the stems was carried out within a period of less than 20 minutes, with light as dim as seemed feasible for accurate handling. The inoculated stems in their tubes were then shielded, as before, by layers of black cloth in the dark cellar room. They were not again exposed, even to dim light, until they were to be tested. From time to time samples of the stems were crushed and their undiluted juices were used to inoculate plants of *Nicotiana glutinosa* L. Increasing numbers of lesions on *N. glutinosa* plants in successive tests furnished evidence of virus increase. The average number of necrotic lesions from 2 tests on the day of stem inoculation was 0; 1 day after inoculation, 1; 2 days after inoculation, 1; 3 days, 12; 5 days, 58; 7 days, 193; 10 days, 503; 12 days, 408; 14 days, 437; and 16 days, 641. These measurements gave evidence that virus did increase, al-

though in this experiment neither chlorophyll nor light was present. The rate of virus increase was comparable to that occurring in green leaves and stems of Green Mountain potato in the course of systemic necrosis induced by inoculating tobacco-mosaic virus into green plants under ordinary conditions of lighting.

In another experiment, using similar methods, a normal or nearly normal rate of virus increase was found in whole tomato plants placed in darkness 1 day before inoculation, and tested subsequently at intervals of 1 to several days. In this experiment chlorophyll was present but light was excluded.

There may remain some doubt as to the adequacy of the experimental conditions for total exclusion of chlorophyll and light. The evidence furnished by these observations leads to the conclusion, however, that tobacco-mosaic virus is able to increase normally under experimental conditions that would ordinarily be considered to exclude chlorophyll, light, and, consequently, the process of photosynthesis.—FRANCIS O. HOLMES, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

*Aphid Transmission of Potato Yellow Dwarf.*—The natural means by which the disease known as yellow dwarf of potatoes is transmitted under field conditions has not yet been determined. The high percentage of yellow dwarf in many commercial seed stocks suggests that some active insect vector is concerned. The virus has previously been transmitted mechanically and is regularly perpetuated through seed tubers. While the disease is recognized chiefly by the yellowing and dwarfing of the leaves, a rusty discoloration of the pith of the stem and tubers is a valuable diagnostic symptom.

Experiments<sup>1</sup> were undertaken in order to test various sucking insects as possible vectors under greenhouse conditions. The usual methods of technique were used. Twenty-five to 50 aphids were transferred from yellow dwarf Irish Cobbler potatoes to each of the plants of the varieties Bliss Triumph, Rural New Yorker, or Irish Cobbler under test.

The inoculated plants together with suitable controls were maintained for observation in a warm greenhouse for at least 6 weeks. The stems were then slit longitudinally and examined for rusty discoloration, which is believed to be a satisfactory diagnostic symptom for current infection.

As shown in the following table, a fairly high average percentage (60.5 per cent) of infection with yellow dwarf was secured with the peach aphid, *Myzus persicae* Sulz., on the 3 varieties of potatoes. The results with the potato aphid, *Macrosiphum solanifolii* Ashm., and with the onion thrips,

<sup>1</sup> Supported by the University of Wisconsin Research Fund.

TABLE 1.—*Transmission of Yellow Dwarf by the Aphid, Myzus persicae Suls*

Variety inoculated	Number of plants tested	Number of plants infected	Number of non-inoculated controls	Number of controls infected
Rural New Yorker . . .	70	57	53	3
Irish Cobbler . . . . .	55	28	27	0
Bliss Triumph . . . . .	55	24	50	0

*Thrips tabaci* Lind., have been mostly negative in the trials conducted thus far. It appears quite probable, therefore, that, under field conditions, the peach aphid may be the active vector of potato yellow dwarf.—KARL KOCH, University of Wisconsin, Madison, Wis.

*Medium for Growth of Pythiaceae Fungi*.<sup>1</sup>—The maltose malt medium described by Leonian, in his study of factors promoting pycnidium formation in some Sphaeropsidales<sup>2</sup> has been used successfully as a medium for the growth of Phytophthora species.

After boiling the ingredients together a short time, a clear amber-color extract may be obtained by filtration. It remains clear during subsequent autoclaving, and also with sufficient phosphoric acid or potassium hydroxide to give reactions from pH 1.5 to 11.5, added aseptically after autoclaving. It is very useful in studies requiring the weighing of mycelium, as well as in detailed microscopic studies.

A modification of Leonian's maltose malt medium, substituting dextrose for maltose, and using easily weighed quantities of the ingredients, to expedite preparation, has given as good growth as has his medium. The modified formula is as follows:  $\text{KH}_2\text{PO}_4$ —1.0 gram,  $\text{MgSO}_4$ —0.5 gram, Bactopeptone (Difco standardized)—1.0 gram, Difco extract of malt, desiccated powder—5.0 grams, dextrose—15.0 grams, water—1 liter. Autoclave at 15 pounds for 30 minutes. For the solid medium, add 15 grams of agar-agar (Difco standardized) per liter.

This medium has been used for 3 years in this laboratory for all stock cultures of Pythiaceae fungi and for saprophytic and parasitic imperfect fungi. These include all species of Phytophthora, Nematosporangium, and Pythium studied by Sideris<sup>3</sup> for which he recommended media prepared

<sup>1</sup> Published with the approval of the Director as Miscellaneous Paper No. 8 of the Pineapple Experiment Station, University of Hawaii, Honolulu, Hawaii.

<sup>2</sup> Leonian, L. H. A study of factors promoting pycnidium-formation in some Sphaeropsidales. Amer. Jour. Bot. 11: 19-50. 1924.

<sup>3</sup> Sideris, C. P. Taxonomic studies in the family Pythiaceae. I. Nematosporangium. Mycologia 23: 252-295. 1931.

from honey dew melon seed, watermelon seed, or papaya fruit. Vegetative growth and reproduction on it are superior to those on corn-meal agar and Leonian's succinic acid medium<sup>4</sup> and equal to that on oat or papaya agars. *Phytophthora* species<sup>5</sup> grown on it readily produce zoospores when transferred to dilute media.<sup>6</sup>

Either Leonian's maltose malt medium or the modification of it, given above, has been more satisfactory than other media ordinarily used for culturing the Pythiaceae, from the standpoint of ease of preparation, ability to precisely duplicate composition, and availability, both as a clear liquid and a clear solid of comparable composition.—F. P. MEHRlich, Experiment Station of the Association of Hawaiian Pineapple Cannerys, University of Hawaii, Honolulu, Hawaii.

<sup>4</sup> Leonian, L. H. Differential growth of *Phytophthoras* under the action of malachite green. *Amer. Jour. Bot.* 17: 671–677. (p. 673). 1930.

<sup>5</sup> While growth of *Phytophthora infestans* occurs on this medium, it has not been compared with growth on other media.

<sup>6</sup> Mehrlich, F. P. Taxonomy and host range and asexual reproduction of *Phytophthora* species causing heart rot of pineapple plants. *Phytopath.* 24: In press. 1934.



## BOOK REVIEWS

Paul Sorauer, *Handbuch der Pflanzenkrankheiten*. Fifth edition. Volume III. Die pflanzlichen Parasiten. Pp. VIII + 948. Paul Parey, Berlin, 1932. Bound, 66 Reichsmarks.

The second volume of Sorauer's manual, which dealt with plant diseases due to bacteria, to slime molds, to phycomycetes, and to ascomycetes, made its appearance in 1928. In the present volume, one of the largest to be issued in this or in any previous edition of the set, are treated the diseases of living plants and the damage to plant products attributable to destructive forms among the basidiomycetes, the fungi imperfecti, the algae, the lichens, and the seed plants. The unexpectedly long lapse of four years between the dates of publication of the two parts concerned with parasites and epiphytes belonging to the plant kingdom is explained in a preface by Appel, one of the editors, as being due mainly to an increasing realization of the need for a complete revision of the earlier edition. Moreover, the deaths of Noack and Hosterman made necessary the appointment of four new collaborators to work up the important section devoted to the fungi imperfecti.

The present volume contains no section corresponding to the chapter on the control and prevention of plant diseases due to fungi that took up 34 of the 310 pages in the corresponding volume of the fourth edition; the matter covered therein evidently being reserved for expanded treatment in a separate volume dealing with plant protection announced forthcoming by the publishers as the sixth and concluding unit in the set. Yet, in spite of this diminished scope, the book under review contains more than three times as many pages as its predecessor of 1923. The more extensive treatment has effected an immense improvement in the utility of every section. The expansion of the section on the rusts from 59 to 132 pages has enabled Köhler to give due attention to the important discoveries on the biology of these parasites made during the last two decades, in large part in the United States and Canada. The section on the Ustilaginales consisting of 147 pages contributed by Zillig, sets forth our knowledge of the smuts with a completeness not approximated in his earlier account of 39 pages. Laubert's treatment of the Exobasidiineae, in 15 pages, is incomparably more instructive than Lindau's earlier 2-page summary. Likewise, the section of 107 pages on the Hymenomycetinae, written by Münch, deals with the pathology of living trees and the biology of timber decay with a fullness impossible in the 17 pages of text contributed earlier by Lindau, much of which necessarily had to be devoted to taxonomic discussion. The 87 pages of the chapter on the Sphaeropsidales by Laubert and Richter have not only

permitted these authors a much fuller textual treatment than was afforded by the 22 pages in the corresponding section of the previous edition, but enabled them to include 31 figures in place of a single figure. Pape's account of diseases due to members of the Melanconiales occupies 83 pages and contains 16 illustrations, in comparison with 25 pages and 3 illustrations devoted to the analogous section of earlier date.

Nowhere has the expanded treatment resulted in more evident improvement than in the difficult section on the Hyphomycetes contributed by Wollenweber. Here, 253 pages, in which the uniformly admirable text is illustrated with 18 figures, replace 57 pages containing 3 figures. Much of the increase is apportioned to several genera whose status in plant pathology has been made much better known in recent times; among which may be cited *Botrytis*, *Alternaria*, *Cercospora*, *Graphium*, *Verticillium* and *Helminthosporium*. The lion's share of the increase, however, went to the chapter on *Fusarium*, to which is devoted a total of 87 pages, or 65 pages more than in the previous edition—an expansion to be accounted for in part, no doubt, by the predilection of the author. That this chapter constitutes virtually a monograph within the more general compendium will make the volume no less attractive to the numerous workers dealing with members of the genus to whom the same author's more extended account may be inaccessible.

A section of 13 pages on parasitic algae deals with a field of phytopathological interest too often ignored in American textbooks. Perhaps the citation here of several substantial papers on *Cephaleuros* and *Rhodochytrium* by American investigators may direct increased attention to the occurrence of algal plant parasites in the United States. A chapter of 10 pages from the pen of Pape disposes of the curious damage to plants due to lichens. The final chapter in the book, written by Köhler, and consisting of 32 well-illustrated pages, deals competently with the more familiar, yet far from commonplace, injuries attributable to parasitic seed plants.

The volume is concluded with an index occupying 50 pages of 3 columns each. Host plants as well as parasites and injurious epiphytes are listed both by their genera and by their common names in German. Diseases are listed mostly by their German designations, but often also by English terms when these have been widely used in the literature. In addition, numerous items are included having reference to discussions on the pathological, morphological, physiological, or biological aspects of particular diseases or of categories of diseases affecting either particular host plants or groups of host plants. References to the literature are given, as in previous editions, in footnotes and in such quantity that if they were assembled they would, in spite of the small type employed for them, fill more than 100 full pages. In thoroughness and documentation the present volume far outstrips not

only the corresponding part of any previous edition but also the companion volume of the same edition; or, for that matter, any compendium whatever covering the same ground. I anticipate there will be little occasion for such expressions of disappointment as were vented by some reviewers of Volume II, when they found omitted all mention of what naturally looms highest to investigators generally—their own individual contributions.

In view of the difficulty of avoiding a catalogue-like style in compositions dealing with innumerable items, the several authors have shown more than ordinary resourcefulness in making the text coherent and readable. The book seems in an unusual degree free from errors in statement, though minor departures from accuracy occur here and there. On page 677, for example, it is stated that knowledge concerning the very inclusive host range of *Helminthosporium giganteum* came to light through “*weiteren Beobachtungen und erfolgreichen Infektionsversuchen*” of mine. In the papers cited in connection with this statement, however, only infections that had come about spontaneously in the field were considered; and certainly no infection experiments of any kind were attempted with the fungus.

The more extended treatment has permitted the inclusion of many illustrations from comparatively recent publications to supplement the older figures hallowed by tradition. The half-tones among these newly introduced illustrations seem of exceptional merit. Some of the zinc cuts, however, lead one to suspect that the hand of the transcribing draughtsman was heavier than it was cunning. Perhaps that hand might have been employed to better effect in wielding scissors and paste-brush since nearly all of the figures in question were coarse enough as published originally to have permitted the necessary reduction in size to be accomplished by direct photographic methods. The depressing character of some other illustrations is hardly to be traced to maladroit transcription, but rather to their failure in the first place to represent properly the morphological features of the fungi they purport to represent. Of the figures assembled on page 835, only two are drawn well enough so that the direction of rotation in the individual sporiferous hyphae can be distinguished. In these two figures both sinistrorse and dextrorse conditions are shown, though the legends indicate that a dextrorse species was concerned in each case. It is undoubtedly in large part through illustrations of such quality that the lingering faith in the transitional position of *Actinomyces* between the bacteria and the fungi, which is reaffirmed in the unhappy sentence opening the special chapter devoted to that genus, is sustained.—CHARLES DRECHSLER, United States Department of Agriculture, Washington, D. C.

*The Helminthosporium Disease of Oats.* By the Department of Plant Husbandry [G. D. O'Brien and R. W. G. Dennis]. The West of Scotland

Agricultural College Research Bulletin No. 3. Pp. 74. With 23 plates and 8 diagrams. Glasgow, 1933.

A rather distinctive feature of the publications that have been issued now for a decade or more from our larger experiment stations in series designated as "Research Bulletins," is that they have permitted extensive discussion of special agricultural problems with regard more particularly to their purely scientific aspects. The present publication, though from a foreign source, is broadly similar to this class of domestic contributions not only in series designation, but in nature of content and in treatment, as well. An introductory section dealing with the "leaf-stripe" disease of oats in Scotland, and a historical review of more general scope, is followed by sections considering in successive order, etiology, growth of the causal fungus in pure culture, morphology of the fungus, duration of viability of its conidia, influence of environmental factors, pathogenicity of the fungus, leaf infection and pathological anatomy, biology of the fungus, control of the disease, and varietal susceptibility. The text is supplemented with appropriate graphs, workmanlike line-drawings, and excellent half-tone reproductions of photographs; and that in numbers so unstinted as to lead one to suspect that the country in which the memoir was published may be unjustly celebrated as a land of excessive frugality. A summary covering one page, and a bibliography citing 70 titles concludes this valuable contribution.—CHARLES DRECHSLER, United States Department of Agriculture, Washington, D. C.

*The Genus Diaporthe Nitschke and Its Segregates.* By Lewis E. Wehmeyer. University of Michigan Studies, Scientific Series, Vol. IX. 11×8 inches. Pp. XII+349. With 18 plates. University of Michigan Press, Ann Arbor, Mich. 1933. Price, \$3.50.

In this book the author, who has published extensively on Pyrenomycetes for more than a decade, undertakes the immense task of bringing order into the welter of more than 650 species that have accumulated in the genus *Diaporthe* since it was erected by Nitschke 67 years ago. Four genera, *Diaporthopsis*, *Apioporthes*, *Diaporthella* and *Cryptodiaporthe*, are recognized in addition to the basic genus, of which an amended definition is supplied. To this redefined genus, cited as *Diaporthe* Nit. Emend., are referred 70 different species not counting the recently described cranberry parasite, *Diaporthe vaccinii*, which is stated in an addendum to be closely related to *D. phaseolorum*. The latter species is definitely made to include as varieties two parasites of economic importance that have enjoyed specific rank under the binomials *D. sojae* and *D. batatis*. The pathogenic form known under the binomial *D. (Phomopsis) citri* likewise is shorn of its independent status, being listed as a variety of *D. medusaea*.

The segregated genera show a smaller, yet still respectable, membership, 19 approved species being listed under *Cryptodiaporthe*, 3 under *Diaporthe*, 8 under *Apioporthes*, and 6 under *Diaporthopsis*. Two species of *Diaporthopsis* are cited as not having been examined, and 1 receives attention in the chapter on "Doubtful species" together with 18 species of *Diaporthe*. Excluded from *Diaporthe* are 89 species, among them the very familiar *Endothia parasitica*, and also the rose-canker parasite, *Diaporthe umbrina*, which is held to be referable to *Cryptosporella*. A final chapter entitled "Species not seen" gives 149 names, all under the genus *Diaporthe*, together with the original descriptions in Latin, English, German, French, Spanish, or Magyar attaching to them.

The morphological criteria are accorded primary importance in distinguishing the various species from one another. Host relationships are, however, kept clearly in view by giving separate treatment to plurivorous forms under each genus or other convenient group of host plants on which they occur. Such separate treatment includes citations of synonyms, individual host species, countries from which the host relationship has been reported, pertinent exsiccati, herbaria or individual collections in which material representative of the host relationship is found, and conidial connections together with appropriate discussion of the fungus in relation to the particular host plant or group of host plants in question. The semi-diagrammatic drawings illustrating outward habits, though hardly elegant, are yet workmanlike and consistent in execution. Especially to be commended are the figures of the spores that appear at a uniform magnification of  $\times 1000$ —a uniformity certainly very desirable in any comparative work. The usefulness of the volume is enhanced by a well conceived index and by adequate keys to the species of the several genera treated. In a period of enfeebled finances it may not be inappropriate to add that the price of the book appears unusually reasonable.—CHARLES DRECHSLER, United States Department of Agriculture, Washington, D. C.

# REPORT OF THE EIGHTEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTO- PATHOLOGICAL SOCIETY

The eighteenth annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of California, Berkeley, June 18-19, 1934, in conjunction with the fourth national summer meetings of the American Association for the Advancement of Science.

The attendance was very satisfactory, there being an average of 50 to 60 visitors at each of the 4 half-day sessions. Over 70 people, most of whom are affiliated with the Pacific Division, signed the register during the meetings.

At a business meeting the following officers were elected :

<i>President</i> .....	C. O. SMITH, University of California Citrus Experiment Station, Riverside, Calif.
<i>Vice President</i> .....	C. W. BENNETT, United States Department of Agriculture, Riverside, Calif.
<i>Secretary-Treasurer</i> ..	B. A. RUDOLPH, University of California Deciduous Fruit Station, San Jose, Calif.
<i>Councilor</i> .....	T. E. RAWLINS, University of California, Berkeley, Calif.

Of these officers, the President will serve one year, the others two.

A total of 44 papers were presented, the titles and abstracts of which follow the present report. The ever-increasing importance of viroses and the attention they are commanding from plant pathologists were exemplified in the large number of papers dealing with diseases of this type that were presented. The balance of the papers reported the results obtained in the study of fungus diseases and subjects pertaining to mycology, nematology, and bacteriology.

Many delightfully informal events punctuated the run of the meetings, not the least of which was the pathologists' dinner. The meal, typically Italian, was served in the evening at a well-known café in Oakland and served to bring the visiting members together under less formal auspices than those of the meetings. Also, many availed themselves of the opportunity to become acquainted with the work being done by the Division of Plant Pathology of the University of California, not only at headquarters in Berkeley, but also at the branch of the College of Agriculture at Davis and at the Deciduous Fruit Station at San Jose.

B. A. RUDOLPH, *Secretary-Treasurer*

*Restoration Properties of Erodium cicutarium on the Attenuated Curly-top Virus.*—C. F. LACKEY.

*Testing Sugar Beets for Resistance to Curly-top.*—N. J. GIDDINGS.

*Properties of the Sugar-beet Curly-top Virus.*—C. W. BENNETT.

*Breeding for Resistance in Blackeye Cowpeas to Fusarium Wilt, Charcoal Rot, and Nematode Root Knot.*—W. W. MACKIE.

The diseases, Fusarium wilt (*Fusarium tracheiphilum*), charcoal rot (*Rhizoctonia bataticola*), and nematode root knot (*Hederodera marioni*), seriously injure Blackeye cowpeas in the interior valleys of California, especially on sandy soils. Varietal tests in infested soil eliminated all varieties, except Iron, Victor, and Brabham, as breeding stocks. Of these, Brabham showed more damage from charcoal rot and nemas than the other two. Victor, while vigorous and resistant, yielded poor seed crops and shattered badly. Artificial crossing between Iron and Blackeye gave the best results. In the  $F_1$  resistance to all 3 diseases was dominant, the seed was black and dappled brown and midway in size between the parents. In the  $F_2$  color, both mottled and self, was dominant to the eye type. The eye type broke up into blackeye, browneye, and smokeye types, with blackeye dominant to the other two. There appeared to be no correlation between early maturity, size and color of seed, and resistance. Correlation appeared to exist between dark-colored leaves and disease resistance. Resistance to the 3 diseases lies in the root cortex, but quality rather than thickness determines resistance. It is suggested that suberin, known to exist in quantity in the Iron parent, an ancestor of Victor and Brabham, accounts for disease resistance. This point is receiving further study. Normal methods of segregation and back crossing to the Blackeye parent have both yielded satisfactory resistant blackeye types that lack only confirmatory tests before being placed in the infested areas.

*A Method of Inducing Knots on Cuttings of the Oleaceae by Bacterium Savastanoi.*—CLAYTON O. SMITH.

Typical knots were produced on cuttings of *Olea europaeae*, *O. chrysophylla*, *Osmanthus aquifolium*, *O. fragrans* and *Forestiera acuminata* when these cuttings were inoculated through wounds with *Bacterium savastanoi*. The method followed was to select wood about a year old and make cuttings 6–12 inches long. The leaves were not removed except at the lower end, which was to go into the fine gravel of the rooting chamber. Three inoculations were made on the lower portion of the cutting, later inserted into the soil, and 2 placed on the upper part of the soil. Control cuttings were similarly treated but without inoculation.

In 4 to 6 weeks no roots had developed on cuttings, but overgrowth  $\frac{1}{4}$ – $\frac{1}{2}$  inch in diameter occurred at the point of injury. These were larger when they were in the soil. The control cuttings in nearly all cases showed no overgrowths. In a small amount of callus tissue formed, it was many times less than the overgrowth on the inoculated cuttings. Artificial inoculations on olives in the open made at the same time as on the cuttings developed typical knots. Inoculated cuttings when placed in gravel under a bell jar also developed knots.

*Spotted Wilt of Head Lettuce.*—C. M. TOMPKINS and M. W. GARDNER.

Heavy losses in the head-lettuce crop are caused by the spotted-wilt virus in certain coastal districts in California. Infected plants are unmarketable or die prematurely.

The disease causes a slight marginal wilting, necrotic spotting, and slight yellowing of the leaves, usually on one side of the plant, with consequent tilting of the head toward the affected side. Lateral curvature of many leaves is produced. Plants may become infected at any age. The necrotic spotting involves even the youngest leaves, and in older heads there may be difficulty in detecting the internal necrosis. Percentage of infection increases with age of crop.

All varieties observed, including Romaine, are susceptible. The virus has been transmitted by the rubbing method followed by an incubation period of 10 to 25 days. The virus from lettuce produced typical spotted wilt in tomato and that from spotted wilt in tomato produced typical symptoms in lettuce. Reciprocal inoculation has been successful between lettuce and a group including eggplant, *Physalis*, pepper, *Nicotiana rustica*, *Salpiglossis*, *Schizanthus* and aster. Local lesions have been produced on snapdragon and broad bean and systemic infection on lettuce by inoculation from calla lily, and lettuce has been infected from spotted wilt in nasturtiums.

*Spotted Wilt of Tomatoes and Its Transmission by Thrips.*—M. W. GARDNER and O. C. WHIPPLE.

Spotted wilt is a serious disease of tomatoes, especially in the cooler coastal regions, and of peppers and head lettuce. It is prevalent in nasturtiums and has been found also in aster, cineraria, *Datura*, petunia, dahlia, calceolaria and calla. The disease has been found in plant beds (tomato, pepper, eggplant, aster) in early spring. The virus has not been transmitted with the seed of tomato, pepper, or *Datura*. It has been transmitted by the rubbing method from tomato to tomato, *Datura*, potato, eggplant, pepper, *Physalis*, *Salpiglossis*, *Schizanthus*, *Nicandra*, *Nicotiana glutinosa*, *N. acuminata*, *N. rustica*, *N. sanderae*, *N. alata*, *N. langsdorffii*, tobacco, petunia, lupine, broad-bean, dahlia, aster, zinnia, chrysanthemum, cineraria, lettuce, *Martynia*, nasturtium, and nettle, and has been recovered from *Datura*, pepper, *N. glutinosa*, tobacco, zinnia, lettuce and calla. Successful transmission by thrips has been obtained between tomato, pepper and *Datura*, from tomato to petunia, from *Datura* to aster, and from cineraria and calceolaria to tomato. In the warmer valleys the earlier necrotic symptoms are followed by stunting, mottling and leaf distortion, while in the cooler regions the necrosis may kill the plants (dieback or streak). Commercial control was obtained in a greenhouse by frequent fumigation and roguing. Infected plant beds are an important source of field infection.

*A Destructive Virus Disease of Cauliflower and Other Crucifers.*—C. M. TOMPKINS.

A serious and widespread virosis of cauliflower (*Brassica oleracea* var. *botrytis*) occurs in commercial plantings throughout the coastal areas of central California. Field losses vary from 20 to 30 per cent, exclusive of losses sustained during transit to eastern destinations. The field distribution of infected plants was general, without suggestion of localized spots. Severely diseased plants were stunted, while the dwarfed, terminal heads were surrounded by small, distorted leaves exhibiting prominent chlorosis, coarse mottling, and necrotic spotting. Chlorosis was generally confined to that part of the lamina between but not adjacent to the midrib or main lateral veins, with small islands of dark green tissue distributed throughout this area. Presumably where late infection had taken place, heads reached marketable size, but enroute to eastern markets the attached, diseased leaves turn yellow, materially reducing the value of the product. Essentially, a low temperature virus, it is readily transmissible by juice inoculations to leaves of healthy cauliflower seedlings. Under greenhouse conditions (55°–65° F.), the incubation period ranges from 12 to 20 days. Successful inoculations also have been



made to cabbage (*B. oleracea* var. *capitata*), and kale (*B. oleracea* var. *acephala*), annual stock (*Mathiola incana*) but not to any plants outside the family Cruciferae. This disease was found in commercial kale seed beds, and it is probable that seed beds may serve as the primary sources of infection of cauliflower seedlings. Roguing of diseased seedlings before transplanting offers a partial method of control.

*Breaking in Stock (Mathiola incana), a Virosis.*—C. M. TOMPKINS.

A systemic virosis of annual stock (*Mathiola incana*) occurs in commercial plantings and home gardens in the coastal areas of central California. The chief symptoms accompanying infection consist of slight to severe stunting of the plant and complete or sectorial breaking of the flowers in the terminal racemes, resulting in a bleached, blotched effect of the undersized petals, occasional mottling of the leaves, and reduction in size of the seed pods. All varieties, particularly American Beauty and Heatham Beauty, in the Giant Imperial and Giant Perfection groups of stock, single and double flowers, including the entire range of floral color from white to red, appear to be susceptible to natural infection. Broken flowers are valueless for the cut-flower trade and, where present, cause a loss. Young, healthy Imperial Crimson stock seedlings with 6 leaves were successfully infected by means of juice inoculations in the greenhouse at approximately 60° F. The incubation period varied from 5 to 7 weeks. Readings usually cannot be taken until the blooming period because of the inconstancy of leaf mottling. Sixty per cent of the inoculated plants became diseased, as proved by the broken flowers, while the non-inoculated controls remained healthy. The relation of this virus to the destructive cauliflower virus and its mode of spread in the field remain to be determined.

*Growing Powdery Mildew on Detached Bean Leaflets and Breeding for Resistance.*—BJARNE DUNDAS.

For use in inoculation tests, the powdery mildew (*Erysiphe polygoni*) of beans, *Phaseolus vulgaris*, was continuously propagated on detached bean leaflets supported on a 10 per cent sucrose solution in Petri dishes stored in the light at room temperature (19°–21° C.). Spores germinated well in dry or saturated air between 15° and 21° C. Field observations and dish tests showed the varieties Pinto, Hungarian, Yellow, and selections from Lady Washington and Pink to be resistant, while Robust, Small White, Red Kidney, Kotenashi, and many others were susceptible. Dish tests with detached leaflets showed that the Pink selection was susceptible when young and that it acquired resistance with age. Necrosis of invaded epidermal cells was associated with resistance. By using the dish test it was found that in the F<sub>2</sub> of hybrids of resistant and susceptible varieties, such as Pinto × Robust, a ratio of 3 resistant to 1 susceptible was obtained. This indicates that resistance to powdery mildew in the varieties tested is due to a simple dominant Mendelian factor.

*Observations on the Basidial Stage of Sclerotium rolfsii.*—J. T. BARRETT.

The basidial stage of *Sclerotium rolfsii* Sacc., apparently has not been reported from the United States. Cultures received in Formosa and Italy from this country have, however, been reported to produce that spore stage. Recently 2 cultures of *S. rolfsii* originating in the United States have produced the perfect stage, which has been compared with a culture of *Corticium rolfsii* (Sacc.) Curzi and with a basidium-producing strain of *Sclerotium rolfsii* Sacc. from New Zealand. Comparison of the vegetative and sclerotial characters were made with a culture of *Corticium centrifugum* (Sev.) Bres. received from Curzi of Italy.

The observations made to date suggest that the 2 cultures from the United States referred to and the New Zealand strain should, at least tentatively, be referred to *Corticium rolfsii* (Sacc.) Curzi.

*A Chytridiaceous Parasite of Phytophthora.*—J. T. BARRETT.

A parasite, a species of the genus *Pleolpidium*, was found in April on *Phytophthora* trapped from greenhouse soil in which snapdragon plants were suffering from a crown rot caused by *Phytophthora cactorum*. Of the few species of *Pleolpidium* described, none has been reported on *Phytophthora*. The parasite produces enlarged, usually spherical, swellings on the hyphae but, of more interest, it infects the sporangia. These cannot readily be distinguished from normal sporangia until the parasite has reached the swarm-spore stage. At the time of maturation of the swarm spores the finely granular character of the contents due to the small size of the swarm spores is seen in contrast to the much larger differentiated swarm spores of the host. Discharge of the small uniciliate swarm spores is through one or more very short hyaline exit tubes, which protrude through the wall of the host sporangium. The empty sporangium thus seen has the papilla of dehiscence of the host sporangium left unbroken.

Successful inoculations of *Phytophthora cactorum* isolated from infected snapdragon plants with the parasite have been made.

*A Bud Blight of Gooseberry Apparently Caused by Nematodes.*—RALPH E. SMITH, H. N. HANSEN and C. EMLEN SCOTT.

A peculiar disease of English gooseberry has been under observation near Albion, Mendocino County, California, for a number of years. The disease is characterized by the nondevelopment of the leaf buds and spurs, or the production of a few deformed, stunted leaves from some of the buds. The lateral spurs remain very much shortened and somewhat swollen and club-like. The whole plant becomes stunted and its yield is greatly reduced.

No cause for this trouble was discovered until the present season, when a generous sample of diseased stems was gathered by a grower during a period of very foggy weather and brought immediately to the laboratory. Examination with the microscope then showed the presence of enormous numbers of nematodes in the diseased buds and also imbedded in the tissue of some of the partially developed leaves. The character, abundance, and location of these worms left little doubt as to their being the cause of the trouble.

No report of a disease of this sort has been found in America. In England, however, a similar disease is described on black currant, caused by *Aphelenchus ribis*. Dr. G. Steiner, Senior Nematologist, U.S.D.A., tentatively considers the Mendocino County material as *Aphelenchoides ribis*, which may prove to be a synonym of *A. fragariae*.

*Quantitative Determinations of Sclerotium rolfsii in the Soils of Sugar-beet Fields.*—L. D. LEACH.

*Sclerotium rolfsii* causes a serious root rot of sugar beets in California. A method of determining the approximate numbers of sclerotia in field soils has been devised. A composite of soil samples taken 8 inches deep from a definite area is dried, pulverized, screened, and passed through a Boerner sampler to obtain 4 400-gram aliquot parts. These are washed separately through a series of 3 screens (10-, 20-, and 40-mesh) under a strong stream of water and the sclerotia are recovered with forceps from a water suspension of the residue. Germination trials reveal the percentage of viability. In 1931 a 70 per cent infection on sugar beets produced a population of approximately

5,000 sclerotia per square foot of soil. Determinations in the same field in 3 successive years following barley plantings showed 1,100, 175, and 40 living sclerotia per square foot. In other fields the decrease following diseased beets has been less rapid. In one field a population of 150 living sclerotia per square foot produced a 20 per cent infection on sugar beets. This method is being used as a direct measure of the efficiency of crop rotations, cultural practices, fertilizers, and fungicides in reducing the infestation in experimental plots and in commercial fields.

*Seed Transmission of Fusarium Yellows of Beans.*—JAMES B. KENDRICK.

In 1929, Harter (Phytopath. 19: 84) briefly described a vascular *Fusarium* disease of field beans (*Phaseolus vulgaris*) observed in the Sacramento Valley, California. The disease was observed by the writer in this locality in 1929 and again in 1933. Bulk seed was harvested in 1933 by crushing diseased pink bean plants in a cloth bag and also by picking pods from diseased plants and opening them by hand so as not to contaminate the seed. This seed was grown in steam-sterilized soil in a greenhouse. Of the 482 plants grown from the bulk seed, 56, or 11.6 per cent, showed wilt, while 481 plants from the hand-picked seed showed no wilt. Seed from the crushed diseased plants was dusted with Semesan at the rate of 2, 4, and 8 ounces and Cerosan at the rate of 4 ounces per 100 pounds of beans and grown in steam-sterilized soil. One hundred and eighty-eight plants from the 2-ounce treatment showed 1 diseased plant; 117 plants from the 4-ounce treatment showed 2 diseased plants; 166 plants from the 8-ounce treatment, and 157 plants from the 4-ounce Cerosan treatment showed no disease. This evidence indicates that the spores of the causal *Fusarium* are carried on the seed coat and are deposited there during the harvesting operations.

*Evidence Suggestive of the Existence of a Filterable Stage of Bacterium tumefaciens.*—

G. E. M. CLARK.

A single strain of *Bacterium tumefaciens* was grown in nutrient broth, and cultures of different ages (2 to 43 days) were passed through a Berkefeld "N" filter. The filtrates were inoculated into *Bryophyllum calycinum*, and also cultured in various ways. Out of 8 tests, 5 filtrates yielded *Bacterium tumefaciens* (pathogenic), though only one was directly pathogenic to *Bryophyllum*.

Similar tests made with milk cultures furnished no certain case of pathogenicity from direct inoculation, though 4 out of 6 filtrates contained *Bacterium tumefaciens*, which was pathogenic. Three tests made with filtrates from crushed crown galls produced only negative evidence.

Two originally identical strains, only one of which was pathogenic to *Bryophyllum*, gave rise to rough and smooth types of *Bacterium tumefaciens* after having been grown for 3 months in litmus milk. In the pathogenic strain, the rough organism was the more virulent.

*Nonsterile Soil Leachate Stimulating to Zoosporengia Production by Phytophthora Sp.*—

F. P. MEHLICH.

By growing *Phytophthora cinnamomi* or *Ph. parasitica* in a maltose malt-extract broth, followed by rinsing in sterile distilled water, and incubation in a nonsterile aqueous leachate of field soil, zoosporengia are produced more consistently and abundantly than by other published methods.

Autoclaving greatly limits the effectiveness of leachate, for the differences in the abundance of sporangia produced in sterile and nonsterile extracts are repeatedly of the order illustrated by Leonian (Amer. Jour. Bot. 12: 450. 1925) as concentrations II or

III and V, respectively. Neither aseptic aeration nor the addition of a trace of  $\text{Fe}(\text{NO}_3)_3$  has increased the effectiveness of autoclaved leachate. Differences between pH of autoclaved and nonsterile extracts have not been correlated with numbers of sporangia produced.

Sterilization of soil, prior to leaching, using Pistor's method (reported to give bacteriologic sterility without markedly affecting solubility of ash, total acid or pH, MgO nitrogen, total nitrogen, or HCl hydrolizable nitrogen) has had the same limiting effect as autoclaving.

Sterile leachate has been superior to sterile M/100  $\text{KNO}_3$  or Petri's solution. Nevertheless, the use of Petri's solution inoculated with nonsterile leachate has given as good a production of sporangia as the original leachate. Thus it appears that factors associated with the presence of microorganisms of field soils are stimulating to copious sporangial production.

Using these methods, papillate zoosporangia of *Phytophthora cinnamomi* have been produced in considerable numbers contrasting with the blunt nonpapillate ones described by all other workers.

*Laboratory Experiments on the Control of Brown Rot of Lemons.*—L. J. KLOTZ, H. S. FAWCETT, and L. L. HUILIER.

Bordeaux mixture, solutions of copper sulphate, and hot water (115°–120° F.) are the most effective means yet discovered for combating brown rot. Each, however, has one or more undesirable features. When followed within 2 years by hydrocyanic acid fumigation, a spray of Bordeaux mixture may cause severe injury to the trees. Copper sulphate in the wash water corrodes galvanized iron tanks and is precipitated if used in combination with soaps and alkaline washes. Hot water causes a liberation of rind oil and the resulting oil spottings when the fruit is cold and turgid.

A search is being made for effective materials not having these undesirable features, 89 different solutions of various chemicals having thus far been tried. The criteria for judging relative effectiveness were: (1) The period of motility of zoospores in a drop of watery suspension placed on a dried film of the material; (2) effect on germination; mounts prepared in (1) were placed in moist chamber and later examined for germination; (3) protection of fruit; to simulate orchard conditions, lemons sprayed and allowed to dry 20 hours were atomized with a zoospore suspension and subsequent infections noted.

The effectiveness of a chemical in wash water was estimated by the number of infections that developed after atomizing the lemons with a spore suspension, leaving undisturbed one hour and then immersing them for 2 minutes.

As a tree spray the most promising substitute yet found for Bordeaux is a mixture of zinc sulphate and hydrated lime, prepared by the usual procedure used in making Bordeaux. The relative efficacy of the 2 sprays is illustrated in one experiment in which 4–4–50 Bordeaux permitted an average of 1.7 infections per fruit, zinc sulphate-lime 3.4 infections, while the average per check lemon was 24.7. The present recommendation is to increase the zinc-lime content to make an 8–8–50 spray.

For use in wash water at packing houses, such mild alkaline washes as 1 to 2 per cent solutions of soda ash, trisodium phosphate, and sodium silicate have given highly promising results, approaching the effectiveness of the standard 1–8000 copper sulphate solution.

*Some Factors Influencing the Character of Interaction Between Trichoderma and Other Soil Fungi.*—R. WEINDLING.

The character of interaction on nutrient media between *Trichoderma* and other soil fungi is determined by various internal and external factors. By varying such en-

vironmental conditions as acidity, moisture, or temperature, the parasitic action of *Trichoderma* may be suppressed and the colonies may show either mutual inhibition or compatibility. When planted opposite *Rhizoctonia* in a Petri dish, *Trichoderma* sometimes will not coil around *Rhizoctonia* hyphae. Direct attack, however, can be induced by sowing *Rhizoctonia* on an agar suspension of *Trichoderma* spores. After a definite period following spore germination in the solidified agar, *Trichoderma* shows maximal parasitic activity.

These phenomena can be connected with the properties of a lethal principle of *Trichoderma*. Probably additional mechanisms are involved, such as staling effects, enzyme production, etc.

Different groups of *Trichoderma* are influenced differently by the above factors. The action of *Trichoderma* is affected also by the host, e.g., in the case of *Pythium*, lethal effect is easily obtained but is rarely accompanied by coiling around the hyphae.

The interaction between members of any pair of microorganisms may thus be of a dynamic nature similar to that shown by mycorrhiza, or smuts on grains, i.e., changes in conditions may transform compatibility between two organisms into an antagonistic or even parasitic relationship.

*Various Fungi Recently Found to be Parasitic on Rhizoctonia solani.*—R. WEINDLING. ✓

Various fungi grown on culture media with *Rhizoctonia solani* attacked the latter in a manner resembling the parasitic action of *Trichoderma*. Under the conditions of these tests, their virulence diminished in the following order: *Acrostalagmus* spp., *Aspergillus niger*, *Penicillium* spp., *Fusarium lateritium*, and *Botrytis cinerea*, *Verticillium* spp. showed only a slight tendency to direct attack. Toxic substances produced by these molds seemed to be instrumental in killing and in preventing growth of *Rhizoctonia* when parasite and host were in actual contact as well as when they were at some distance from each other.

Thus it appears that many soil saprophytes are capable of this peculiar type of parasitic action. Various common molds are reported to be able to make use of dead microorganisms in their metabolism. Perhaps one of the reasons that they are so common in the soil lies in their ability to overcome their competitors in the struggle for food and eventually use the dead hyphae as nutrients.

*The Use of Nitrogen Trichloride and Other Gases as Fungicides.*—L. J. KLOTZ.

Nitrogen trichloride gas, in concentrations lethal to fungi of decay but not injurious to citrus fruits, is being employed with much promise to protect fruit in storage rooms and in loaded cars. Equivalent concentrations of chlorine, while more toxic to the fungi, injure the rind of fruit and thus prepare the way for greater losses. Ozone is but slightly inhibitory to the fungi and the rate of decay in inoculated oranges.

Concentrations of  $\text{NCl}_3$  gas as low as 4 to 6 milligrams per cubic foot for a period of 30 minutes were lethal to heavy plantings of conidia of *Penicillium digitatum* and *P. italicum* on filter paper and agar. Mycelium of *Phytophthora citrophthora* and spores of *Colletotrichum gloeosporioides*, *Alternaria citri* and *Dothiorella gregaria* were similarly sensitive, but large clumps of mycelium were not penetrated and killed by these low concentrations of the gas. Microscopically, the protoplasts of gas-treated spores, germ tubes, and hyphae were seen to be shrunken and pulled away from the cell walls, and, in some cases, with marked granular and coagulation effects.

After 8 to 10 weeks' storage, losses in oranges due to decay may, as shown by numerous tests, be reduced 50 to 75 per cent or more by giving the fruit 3 to 5 three-hour treatments of 15 mg.  $\text{NCl}_3$  per cubic foot at 3- or 4-day intervals, beginning the first day of storage.

*A Survey Concerning a Native Pathogen, Armillaria Mellea.*—J. LEE HEWITT.

In a highly developed citrus area and the watershed back of it, *Armillaria mellea* was found native and of wide distribution in infection centers that are locally isolated on hillsides and related to flood plains along stream beds. Hosts on hillsides are the oak, and along streams include the willow, poplar, and sycamore.

Distribution from natural habitat to groves is by flood water that deposits infected débris together with infectable débris among stream bank growth, which picks up and transmits the infection by its roots to near-by groves. Invasion of grove areas was more frequent and rapid formerly, in grapes, apricots, and walnuts than now, when citrus is predominant.

Oak, sycamore, and citrus seem resistant. Herbaceous plants were found infected.

Removal of stream-bed growth may retard dispersal.

Control efforts in citrus groves can take advantage of host characteristics and factors of fungus distribution. Treatment by carbon bisulphide or surgical removal may be used.

*Experiments in Biological Control of Rhizoctonia Damping off.*—R. WEINDLING and H. S. FAWCETT.

Experiments to protect citrus seedlings from *Rhizoctonia* damping off in sterilized soils by inoculating the latter with *Trichoderma* were more successful with some types of *Trichoderma* than with others and more so at acid than at neutral reactions. The interactions of the 2 fungi in sterilized soils paralleled those on culture media.

Attempts were then made to utilize this experience for biological control of the disease in nonsterilized soils. At moderately acid reactions soils inoculated with several *Trichoderma* types often gave considerable control, and the *Rhizoctonia* checks showed nearly total loss. In very acid soils almost complete control was obtained. The very acid *Rhizoctonia* checks showed, however, little and sometimes no damping off. Isolation from these *Rhizoctonia* checks proved that *Trichoderma*, which had not been purposely introduced, as well as some other fungi, increased in abundance with increasing acidity, while it became difficult or impossible to reisolate *Rhizoctonia*.

The growth of *Rhizoctonia solani* declines at very acid reactions. In sterilized soils, however, it attacks citrus seedlings in the whole range of reactions used above. The control of *Rhizoctonia* damping off through soil acidification may thus be due primarily to the increased parasitic, antagonistic, and competitive activities of other soil organisms, *e.g.*, *Trichoderma*.

*Tobacco Mosaic on Spinach.*—LEON K. JONES.

The common tobacco-mosaic virus (No. 1) has been used to inoculate Bloomsdale Savoy spinach. Mechanical juice inoculation has given from 50 to 90 per cent infection on spinach plants incubated 16 to 20 days. The first symptom of the disease is a mottling of the older foliage with slightly raised dark green areas in the lighter green tissue. As the affected plants age, increased puckering of the foliage with dwarfing of the plants is noted. The older mottled leaves become yellowed and may die a month to 6 weeks following inoculation.

*Heterothallism in Corn Rust.*—R. F. ALLEN.

*Puccinia sorghi* is a heterocious, heterothallic rust alternating between corn and oxalis. The gametophytic, unisexual mycelium on oxalis produces spermogonia, stomatal hyphae, and small, rapidly deteriorating, haploid aecia. Only when the 2 sexes meet do fertile aecia develop. When spermatia of one sex are transferred to the surface

of an infection of opposite sex, a spermatium placed in contact with a paraphysis or a stomatal hypha becomes attached, its nucleus passes over into the hypha and moves in through it to the internal mycelium. Under favorable conditions a spermatium not in contact with any surface hypha can germinate and form a slender hypha that enters the nearest stoma and so becomes effective. Spermatial nuclei entering gametophytic hyphae divide rapidly and migrate through the hyphae. Twenty-four hours after spermatization 60 per cent of the vegetative cells throughout the mycelium contain more than one nucleus. The haploid aecia also are rapidly diploidized and then progress to spore formation. One fertilization suffices for a lifetime. All aecia forming after the fertilization will mature normally.

*Heterothallism in Flax Rust.*—R. F. ALLEN.

*Melampsora lini* (flax rust) is autoecious and heterothallic. The gametophytic, unisexual mycelium, characterized by cells containing 1 to several nuclei, produces spreading spermogonia with few, weak, irregular paraphyses. There are few or no stomatal hyphae, and doubtful aecial anlage. When spermatia of one sex are transferred to the surface of an infection of opposite sex they enter the leaf through epidermal cells or through spermogonia, grow for a brief time in the leaf tissue, and then pair with the native hyphae in the young aecia to form "two-legged" basal cells that give rise to chains of aeciospores.

*The Parasitic Action of Omphalia sp. on Tissues of the Date Palm.*—DONALD E. BLISS.

Lesions produced by *Omphalia* sp. constitute a specific, diagnostic symptom of decline disease, and they may be found in all underground portions of affected trees. When placed in soil about healthy plants, the fungus first becomes established in dead portions of the outer leaf bases. Rhizomorphic strands then penetrate to inner layers of leaves and grow along root surfaces where mats of mycelium become closely attached to the epidermis. Underlying cells are killed, their walls darken, and often collapse, before fungous invasion begins. In leaf tissue the starch grains disappear from cells at the margin of the advancing lesion. Apparently the fungus enters wound-free tissues, penetrating cell walls and filling all cavities, including the vascular elements, with closely woven masses of hyphae. Although small at first, lesions may enlarge gradually until entire sections of roots or leaf bases are killed. The abortion of young primary roots as they penetrate diseased leaf bases to reach the soil, constitutes the most damaging effect on the palm. Lesions 2 cm. in depth were observed in trunk tissue of old palms that were severely stunted and in that of young seedlings and immature offshoots. In the latter cases death resulted from fungous invasion of the terminal bud.

*A Case of Almond Blossom Infection by Coryneum beijerinckii Furnishing Inoculum the Following Year.*—E. E. WILSON.

Twig lesions are regarded as the most important means by which *Coryneum beijerinckii* passes from one season to the next. Examinations during the spring of 1934, however, showed the fungus sporulating on mummied blossoms that had been blighted the year before. It was further found that *C. beijerinckii* attacked the calyces during anthesis in 1934, and that in a few cases the lesions spread downward to the pedicel, blighting the blossom. Whether *C. beijerinckii* commonly blights almond blossoms remains to be proved. The great number of mummied blossoms in the orchards under observation may have first been attacked by *C. beijerinckii* and later blighted by the brown-rot fungus, which also was common.

Aside from the fact that *Coryneum beijerinckii* may pass from one season to the next, in certain cases, on dried-up blossom parts, is the equally important fact that calyx lesions constitute sources from which conidia may spread, after bloom, to the young fruit and leaves. It was observed that the calyces, even after abscission from the receptacle, adhered to the developing fruit and that numerous lesions subsequently developed on these fruits.

*Localization of Symptoms During the Early Stages of Curly-top Infection in the Sugar Beet.*—KATHERINE ESAU.

Phloem degeneration and the external symptoms of curly-top of the sugar beet become perceptible first in those parts of the plant nearest the inoculated leaf and directly connected with it by phloem tissue. This suggests that the virus is translocated in the phloem. Xylem seems not to be concerned in this movement, since phloem degeneration occurs in those traces where no xylem has yet differentiated.

*The Rate of Spread of the Veinbanding Virus on Potatoes.*—LEON K. JONES.

In the summer of 1933 plantings of from 2 to 13 hills each of 31 virus-free seedling strains and  $\frac{1}{4}$  acre of virus-free Early Rose potatoes were made near Pullman, Washington, at least  $\frac{1}{2}$  mile from soil that had ever produced potatoes. The potato planting was surrounded by fields devoted to barley and alfalfa. The potato plants became naturally infected with the veinbanding virus during the growing season as follows: Thirteen seedling strains, 100 per cent; 6 seedling strains, over 50 per cent; Early Rose, 59 per cent; 5 seedling strains, 10 to 50 per cent; 7 seedling strains remained virus-free.

*Types of Trichoderma Rot of Lemons and Oranges.*—H. S. FAWCETT and R. WEINDLING.

The observations here recorded are based on inoculations made with 30 cultures of *Trichoderma* into wounds of mature lemons. The typical *Trichoderma* rot was described by Fawcett and Lee (*Citrus Diseases and Their Control*). It was always produced by cultures of *T. lignorum* having a coconut-like odor. This rot never was found with the nonodorous type of the species that is able to produce a yellow pigment. In a few cases this second type invaded the core of the fruit several weeks after inoculation, induced an internal rot, and finally broke out on the rind. A third type of *Trichoderma*, nonodorous and nonpigmented, caused a rot that differed from the typical one by showing a much lower percentage of infections, a firmer appearance, and very slow development. Using smaller numbers of cultures and fruits, the results obtained by inoculating mature Valencia oranges with these 3 types of *T. lignorum* were similar to those on lemons.

Some single-spore cultures from an odorous isolation of *Trichoderma lignorum* grew very slowly. They produced but little of the characteristic odor and showed no parasitism on lemons.

A culture of *T. Koningi* was found to cause a rather soft, easily punctured buffy brown rot.

*Interspecific Anastomosis and the Origin of New Types in Imperfect Fungi.*—H. N. HANSEN and RALPH E. SMITH.

Two species of *Botrytis* (*B. allii* and *B. ricini*) were observed to anastomose when grown together in co-cultures. Their progeny was analyzed through many culture series by single-spore methods. Of 20 single-spore isolations from the original mixed culture, 6 were identical with one parent, 9 resembled the other, while 5 were



decidedly different from either. By repeated single sporing from these aberrant cultures 3 types were segregated that appeared to be sufficiently distinct to be considered new varieties or even new species. These types have remained constant through 3 single-spore-culture series. Contemporaneous series from the original parents showed no variation. The suggestion is offered that the production of these aberrant forms from combined, interspecific cultures of imperfect fungi may result from genic changes induced by the presence of specifically different nuclei in the same cell brought together by the mechanism of anastomosis.

*Dematophora Root Rot.*—HAROLD E. THOMAS, H. N. HANSEN and H. EARL THOMAS.

This disease, first recognized as distinct from other root diseases about 5 years ago, is killing trees in a number of apple orchards in Santa Cruz County, California, and in at least one apricot orchard in Alameda County. The symptoms and associated fungus agree in essentials with published descriptions of the root rot caused by *Dematophora necatrix*. Pathogenicity of the fungus has been demonstrated with small apple and pear trees.

The disease may be distinguished from those caused by *Armillaria* and *Xylaria* by the absence of well-defined rhizomorphs or stromata and the presence of profuse, white, cottony mycelium adjacent to affected roots in moist air in the laboratory or in soil cavities in the orchard. Coremia of *Dematophora* are found occasionally near the ground line on trees killed by the disease. They usually are formed also in pure cultures of the fungus on steamed kernels of wheat and oats.

*The Relation of Carbon-nitrogen Ratio and High Acidity to Color Production by Fusarium Species.*—A. W. DIMOCK.

As the result of studies of the relation of the carbon-nitrogen ratio and the acidity of the substrate to the production of pigment by *Fusarium* species the following conclusions were adduced:

1. The factors affecting color production in the genus *Fusarium* differ with the species and even the strain involved.
2. The production of nondiffusible pigment by a gladiolus *Fusarium* was found dependent upon a very high acidity of the medium. The production of diffusible pigment by this organism was, however, conditioned by an actual deficiency in nitrates, the reaction of the substrate being only of indirect importance.
3. The production of diffusible pigment by *Fusarium lycopersici* var. *pisi* required a carbon-nitrogen ratio of greater than 1, but was not dependent on a deficiency in nitrates. The hydrogen-ion concentration seemed important only as it affected the growth of the organism.
4. It was seen that not only do the carbon-nitrogen requirements for pigmentation vary with different *Fusarium* species, but the nature of the required carbon source also varies.

*Canker on Chamaecyparis lawsoniana.*—J. W. HOTSON and D. E. STUNTZ.

Frequently, there has been brought to the attention of the nurserymen in the vicinity of Seattle a disease occurring on *Chamaecyparis lawsoniana*, involving the formation of a stem canker that eventually kills the tree by girdling. The cankers usually are produced on the stem just above ground level, or on the root collar, rarely higher on the trunk, apparently resulting from infection through wounds. This disease causes losses of Lawson cypress nursery stock and has been the subject of investigation with the object of isolating the responsible organism.

From the canker on the stem of a small *Chamaecyparis lawsoniana* obtained from a local nursery, there has been isolated what apparently is a species of *Monochaetia*, believed to be responsible for the production of the canker. By means of inoculation of a number of trees of *Chamaecyparis lawsoniana* with the organism grown in pure culture, definite proof has been obtained that it is parasitic, capable of killing small seedlings outright, and of producing cankers on stems and branches. From the disease thus produced artificially the organism was reisolated. Further work is anticipated to establish definitely the identity of the organism and determine the extent and nature of the damage it is capable of causing.

*The Use of Oil-soluble Copper as a Fungicide.*—E. R. DE ONG.

It has been found possible to carry minute amounts of copper into the tissue of leaves and twigs by the use of copper resinate dissolved in a specially prepared pine-tar oil known as Palustrex. Such application offers the possibility of placing copper in contact with the mycelium of fungi after penetrating into plant tissue. Copper has been recovered in measurable amounts from the interior of treated twigs where practically no copper was found on the surface after three weeks exposure. This in contrast with a standard Bordeaux, where 83 per cent of metallic copper was recovered from the surface of leaves and twigs three weeks after application, but with the methods followed none could be detected on the interior of either leaf or twig. The copper resinate is supplied in the form of a heavy paste and dissolved in the Palustrex Oil, which had been especially treated to make it safe for foliage applications. Results to date indicate the possibility of control with much smaller amounts of copper applied in this way than for surface applications of such a material as Bordeaux spray.

*Downy Mildew of the Hop in California.*—C. EMLÉN SCOTT and H. EARL THOMAS.

The hop downy mildew, caused by *Pseudoperonospora humuli*, was recognized for the first time in California on May 1, 1934. The wide distribution of the fungus in Sonoma and Mendocino Counties indicates that it is of remote introduction into this State. Local leaf infections, spike formation of terminal growth, and nodal infections as described by earlier workers are present in abundance.

The disease was found in severe form in escaped plants that are, however, seldom found, except in the immediate vicinity of hop yards. No seedling hop plants have been seen.

In 2 distinct infection periods during late May and June, the apparent incubation time for leaf spots was 7 to 9 days, and 9 to 11 days for the production of spikes. These periods were marked by rains preceded and followed by relatively dry weather.

There is some evidence to indicate that considerable resistance is developed by stems with increasing age.

*Control of Soil Fungi by Fumigation with Chloropicrin.*—G. H. GODFREY.

Fumigation tests were conducted with chloropicrin against various plant-parasitic soil fungi, including *Fusarium* sp. from gladiolus, *Verticillium albo-atrum* from strawberry, *Phytophthora cactorum* from snapdragon, *Rhizoctonia solani* and *Sclerotium rolfsii* from sugar beet, and the root rotting fungi *Armillaria mellea* and *Dematophora* sp. Four-gallon glazed stone jars, filled with autoclaved greenhouse soil, were used. Cultures of the fungi were introduced in small glass cells, containing slightly moistened soil wrapped with cheesecloth. These were thrust into the soil adjacent to the sides of the jars and about half way down. There they were allowed to incubate for 24 hours. Chloropicrin was inserted to a depth of 3 inches in the center of each jar, at the rate of

1½ cubic centimeters per jar (equivalent to 400 pounds per acre-foot). The jars were sealed with a glue-coated paper cover. The cultures were removed after 48 hours and plated on agar. All fumigated cultures were completely negative for growth. All control cultures, treated identically, except for the gas, gave good growth.

*New Economic Hosts of the Stem and Bulb Nematode.*—G. H. GODFREY and C. EMLEN SCOTT.

Salsify, *Tragopogon porrifolius*; parsley, *Petroselinum hortense*; and celery, *Apium graveolens*, are reported as hitherto unrecorded hosts of the stem- and bulb-infesting nematode *Anguillulina* (Tylenchus) *dipsaci*. Diseased salsify plants, submitted for diagnosis in the spring of 1933, from San Juan, San Benito County, California, were found infested by this nematode. Symptoms in salsify were swollen leaf bases and "tulip-root" effect in young shoots, with some necrosis in the crown region. Failing near-by parsley plants of the double and triple-curved varieties and Hamburg, a fleshy-rooted variety, all showed typical symptoms of stem-nematode infestation. Symptoms were dwarfing of leaves and leaflets, tulip-root effect with occasional cracking in leaf petiole bases, and occasional rotting off of leaves at the crown. Nematodes identified as *A. dipsaci* were found in petioles and leaflets. In addition there was found extremely heavy infestation within the fleshy roots. This constitutes a new type of injury caused by this organism. The salsify and parsley followed a crop of garlic, *Allium sativum*, known to have been heavily infested with the stem nematode.

The third new host, celery, was not found in the field, but as a result of greenhouse inoculation with colonies of nematodes from garlic and parsley.

*The Use of Carborundum as an Abrasive in Plant-Virus Inoculations.*—T. E. RAWLINS and C. M. TOMPKINS.

Spotted wilt of tomato, *Lycopersicon esculentum*, and lettuce, *Lactuca sativa* L., virus diseases of cauliflower, *Brassica oleracea* L., var. *botrytis* L., broad bean, *Vicia faba* L. var. *minor*, and stock, *Mathiola incana* R. Br., are difficult to transmit by the ordinary methods of mechanical inoculation. These diseases may be readily transmitted by the use of the following technic. Powdered carborundum (370 grain), contained in a salt shaker, is dusted over the upper surface of the leaves to be inoculated. A piece of absorbent cotton, held in forceps, is dipped in virus that has just been extracted from young plants showing the first symptoms of the diseases. The virus-soaked cotton is then rubbed lightly over the carborundum-coated leaves. It is supposed that the sharp particles of carborundum penetrate the epidermal cells without killing them and allow the virus to enter.

*Soil Treatments for the Control of Cotton Root Rot Caused by Phymatotrichum omnivorum.*—R. B. STREETS.

One of the most serious aspects of the whole root-rot problem is the destruction of many valuable long-lived perennials, such as deciduous fruit trees, nut trees, and ornamental trees and shrubs. Our results indicate that heavy applications of ammonium sulphate or ammonium hydrate will prove successful in checking root rot in some tree crops. Outstanding success has resulted from treatment of pecan trees in the Yuma valley. In one orchard no treated trees were lost; in the second grove about 40 per cent of the treated trees died while there was a 100 per cent loss of nontreated trees. Experiments on deciduous fruit trees have been complicated by the great variability of soils in the orchards treated, but results have been mainly encouraging. The use of organic manures, either of high or low nitrogen content, in combination with the above

treatments appears promising. Since both ammonium compounds, especially the hydrate, are very toxic to the trees if applied in too great concentration, a special method was evolved for safe and accurate application under field conditions. While experiments have been conducted largely with badly diseased trees, it is obvious that the effect of treatment would be much more valuable if applied to trees only slightly affected or standing in the path of the advancing fungus. Such trees might be maintained without loss of production, while badly diseased trees must usually be severely pruned, entailing a loss of one or more crops.

*Some Factors Influencing the Sporulation of Certain Species of Phytophthora.*—L. J. KLOTZ and H. S. FAWCETT.

Mycelium of *Phytophthora citrophthora*, *P. parasitica*, and *P. cactorum*, grown in prune extract, produce abundant, normal sporangia when transferred to and kept in a shallow layer of water permitting some of the hyphae to protrude above the water level.

Cleavage of sporangial protoplasm and discharge of sporangia are then readily induced by exposure to two temperatures, 28°–30° C. for 3 to 5 minutes, followed by 15°–18° C. for 1 hour. Swarming begins in a few minutes and reaches a maximum in approximately an hour under these conditions. With *Phytophthora citrophthora*, exposure only to light (artificial or sunlight), or to smaller depressions in temperature, as from 28° to 23° C., in either light or darkness, also stimulate swarming, but the above procedure consistently yields abundant zoospores.

Zoosporangia of *Phytophthora citrophthora* from mycelium grown in glucose potato broth differ from those developed on mycelium from prune extract. In general, they are smaller, fewer in number, form more slowly (first evidence after 5 or 6 days at 20°–22° C., whereas those from prune juice appear as early as 36 hours after transfer to water) and they divide into zoospores less readily, frequently discharging the protoplasm abortively by plasmoptysis.

Mycelium from both media, used full strength (prune extract: 60 gm. pulp per liter; glucose potato broth: 20 gm. dextrose and 250 gm. sliced potatoes per liter) was more abundant and grew more sporangia than mycelium from the media diluted to  $\frac{1}{2}$  concentration. Cultures grown 1, 2, and 4 weeks, respectively, produced similar numbers of zoosporangia when transferred to water.

Sporangia appeared earlier on mycelium from acid media (pH 4.08–pH 6.25) than that from alkaline media (pH 7.9–8.4), although the ultimate numbers were similar, the fungus changing the reaction of both alkaline and acid sets to the acid range, pH 4.16–pH 4.92.

*Physiologic Specialization in Phytophthora Species.*—F. P. MEHRlich.

From experiments to determine the relationship of diseases of other economic plants to pineapple heart rot, evidence was obtained that was indicative of physiologic specialization in species of *Phytophthora*. The cultures tested were separated into 3 categories: (–) incapable of causing heart rot, (+) slightly pathogenic, and (++) virulently pathogenic to the pineapple plant. These are listed below by species, source and number of cultures tested: *P. cambivora* from chestnut 2 cultures –, host unknown 4–; *P. cinnamomi* from cinnamon (cultures of parallel history) 1–, 1+, 1++; from pineapple (Australia) 1++, (Hawaii) 7++; from *Rhododendron* 1+; from chestnut (S. Europe) 1–; walnut (Australia) 1–; *P. palmivora* (*P. arecae*) host unknown 1–, 2+; (*P. faberi*) host unknown 2–, from coconut 1+; (*P. meadii*) from Hevea 1++, pineapple 1++, (Cacao group) from papaya 1–; *Ricinus* 1–, coconut 1–, Palmyra 1–, host unknown 1++; (Rubber group) from coconut 2–, 1+, Hevea 1+; *P. parasitica* (*P. melongenae*) from egg

plant 1++, *Antirrhinum* 1++; from potato 2++, tomato 1++, rhubarb 1+, host unknown 2++.

These data support similar findings by Leonian, Tucker, and Ashby, who used other species and other hosts. They cast considerable doubt on the validity of separating species on the basis of pathogenicity. Such a separation of *Phytophthora cambivora* and *P. cinnamomi*, made on the basis of the differential susceptibility of potato tubers to their attack, seems highly questionable.

*Some Host Responses in Graft Transmissions of Dieback Streak of Tomatoes.*—MICHAEL SHAPOVALOV.

Mechanical transmission of this form of streak was obtained with *Nicotiana tabacum* and *N. glutinosa* in addition to tomato and *Datura stramonium*, as previously reported. *N. glauca* gave no definite results; a faint leaf mottling or a few necrotic spots appeared in some cases but the virus was not recovered. All these hosts were used also in grafting experiments. All grafts were made by the cion method, using diseased cions for healthy stocks and healthy cions for diseased stocks. Successful unions, as a rule, brought about the transmission of the disease to susceptible hosts. Symptomatalogical responses in such cases were similar to those obtained in the juice transmission. Graft-inoculated tomato, tobacco and *N. glutinosa* developed severe streak necrosis. Healthy *Datura* grafted with either of these diseased plants developed blotchy, puckering, coarsely-mosaic leaves and no necrosis. These mosaic *Datura* plants grafted with healthy tomato, tobacco, and *N. glutinosa* brought about the development of typical dieback streak in these susceptible hosts. Healthy *N. glauca* grafted with streaked tomato, tobacco, and *N. glutinosa* plants occasionally developed scarce circular necrotic spots on older leaves, but otherwise remained symptomless. Tops of such *N. glauca* plants grafted with healthy susceptible plants transmitted no disease.

## VI. INTERNATIONAL BOTANICAL CONGRESS

Amsterdam, September 2-7, 1935

The Organizing Committee of the VI. International Botanical Congress announces that the following topics preliminarily have been chosen for discussion in the sections:

**AGR. Agronomy.** 1). Interactions between roots and soil; interactions between plants. 2). Virus diseases. 3a). Weed flora as an indicator of soil conditions in agriculture. 3b). Grassland associations. 4a). Genetics and breeding of immune varieties. 4b). Inbreeding. 5). Importance of microbiological investigations in the study of agricultural problems. 6). Influencing the cycle of development in plants.

**CYT. Cytology.** 1). Structure of chromosomes. 2a). Crossing-over versus conversion. 2b). Terminology of cytology and genetics. 3a). Pairing of chromosomes in polyploids. 3b). Reduction division in fungi. 4). Chain- and ringformation of chromosomes. 5a). Submicroscopic structure of the cell wall. 5b). Vacuome, chondriome, plastids. 6). Colloid chemistry of protoplasm; vital staining.

**GEN. Genetics.** 1a). Experimental mutations. 1b). Genetical basis of size and form. 2a). Crossing-over versus conversion. 2b). Terminology of cytology and genetics. 3a). Sexuality in fungi. 3b). Reduction division in fungi. 4a). Genetics and breeding of immune varieties. 4b). Inbreeding. 5). Taxonomy and genetics. 6a). Plasm and genotype in their mutual relations. 6b). Lethal factors.

**GEO. Geobotany, ecology and phytogeography.** 1). Climax associations in northwestern Europe and North America. 2). Cartography: a). Vegetation maps; b). Area maps. 3). Flora and vegetation area. 4). Plant geography in younger formations. 5). The halophyte problem. 6a). Classification and nomenclature of vegetation units. 6b). Miscellaneous papers.

**MOR. Morphology and anatomy.** 1a). Size and form. 1b). Genetical basis of size and form. 2a). Phytohormones; general paper. 2b). Leaf arrangements. 3). Flower morphology. 4). Female fructification and phylogeny of Conifers. 5a). Wood anatomy. 5b). Relations between anatomy and external morphology. 6). Morphology of bryophytes.

**MYC. Mycology and bacteriology.** 1). Differential characters in Hymenomycetes. 2). Nomenclature of fungi. 3a). Sexuality in fungi. 3b). Reduction division in fungi. 4). Biologic forms of fungi. 5). Importance of microbiological investigations in the study of agricultural problems. 6). Phylogeny and taxonomy of Phycomycetes.

PATH. Phytopathology. 1). Biological basis of plant quarantine. 2). Virus diseases. 3). Various papers. 4). Biologic forms of fungi. 5). Immunization. 6). Physiologic diseases.

PB. Palaeobotany. 1). Geobotanical provinces in the older formations. 2). Caytoniales and Pteridospermae and the evolution of angiosperms. 3). Flower morphology. 4). Plant geography in younger formations. 5). Synchronism and uniformity in palaeozoic and mesozoic floras. 6). Various papers.

PH. Plant physiology. 1). Photosynthesis. 2a). Phytohormones; general paper. 2b). Phytohormones; various papers. 3). Oxidation, reduction and metabolism. 4). Permeability and the accumulation of mineral elements. 5a). Submicroscopic structure of the cell wall. 5b). Translocation of plastic materials. 6). Influencing the cycle of development in plants.

SYS. Taxonomy and nomenclature. 1). Various papers. 2). Caytoniales and Pteridospermae and the evolution of angiosperms. 3). Flower morphology. 4). Female fructification and phylogeny of conifers. 5). Taxonomy and genetics. 6). Phylogeny and taxonomy of Phycomyces.





# PHYTOPATHOLOGY

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## STUDIES ON A LETHAL PRINCIPLE EFFECTIVE IN THE PARASITIC ACTION OF *TRICHODERMA LIGNORUM* ON *RHIZOCTONIA SOLANI* AND OTHER SOIL FUNGI<sup>1,2</sup>

R. WEINDLING<sup>3</sup>

(Accepted for publication January 8, 1934)

### INTRODUCTION AND OUTLINE

In earlier papers (39, 40) the parasitism of *Trichoderma lignorum* (Tode) Harz on *Rhizoctonia solani* Kühn and on other soil organisms has been described. An investigation of the nature of this peculiar action has been started, using the same pigmented strain of *Trichoderma lignorum* and the strain of *Rhizoctonia solani* isolated from Citrus seedlings.

The principal object of this paper is to present evidence for the following fundamental aspects of the action:

1. The parasitic activity of the *Trichoderma* is made possible by a deadly substance, a lethal principle, which is excreted into the surrounding medium by the young hyphae, in presence or absence of other organisms. The time of the highest production of the lethal principle in absence of *Rhizoctonia* is in agreement with the period of greatest parasitic activity during the life cycle of the fungus when grown with *Rhizoctonia*.

2. The lethal principle is very unstable, especially at low hydrogen-ion concentrations. Its effect decreases, therefore, with decreasing hydrogen-ion concentration.

3. The pH ranges of most rapid decomposition of the lethal principle and of its decreasing effect on *Rhizoctonia* agree with the ranges at which little or no parasitic activity is displayed by *Trichoderma* when grown in conjunction with *Rhizoctonia*.

Methods and experiments leading up to the present work have been described elsewhere (41). Parts of this report are included here.

<sup>1</sup> Paper No. 296, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

<sup>2</sup> A portion of this research has been submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Plant Pathology in the Graduate Division of the University of California.

<sup>3</sup> The writer here gratefully acknowledges the continuous interest and advice of H. S. Fawcett under whose direction this work was done. Thanks are due L. J. Klotz for many helpful suggestions.

THE LETHAL PRINCIPLE<sup>4</sup> AND ITS PRODUCTION

## Review of Literature

Most of the literature on toxic substances produced by plant-pathogenic fungi is concerned with the wilts caused by different species of *Fusarium*. The usual procedure has been to grow the fungus in a rich nutrient medium, usually Richards' medium, for several weeks. The criterion for the toxic action of the filtrate obtained from these cultures is the wilting of cut plants or leaves placed into them. The following results have been obtained concerning the properties of these toxic principles of *Fusarium* spp. Most investigators find them heat-resistant (2, 10, 28, 31, 34, 42, 43). Some obtain colloidal (28, 42), others crystalloidal substances (17, 34, 42). There also is conflicting evidence as to partial volatility (20, 31, 34, 42, 43) and to the influence of pH (2, 10, 43). Usually, the toxic products lack the specificity displayed by the fungus (2, 4, 10, 23, 28, 43). In some cases, however, there is a similar reaction of susceptible and resistant varieties of the host plant to the pathogenic fungus and to its toxic principle (18, 31, 42).

Only two authors seem to have made an attempt to exclude the staling products of fungus cultures. Brown (5) tested the lethal principle of *Botrytis cinerea* by microscopic observation of its action on the cells of beet disks, while the macerating principle was examined macroscopically. The two principles seem to be inseparable. They have the character of an endoenzyme. Bewley (1), obtaining an "exoenzyme" from a *Verticillium* sp. causing "sleepy disease," finds its effect almost, but not entirely, destroyed by boiling. Brown (5) criticizes former work in which the killing effect of *Botrytis* has been ascribed to substances produced in culture media on which the fungus had been grown for some time. In these old solutions, staling substances are present that may not be produced by the young and actively growing fungus, and yet exert a certain toxic effect on the plant tissue. Perhaps this also will explain some of the contradictory results of workers with the *Fusarium* wilts that have been cited above. Brown and Bewley are apparently the only workers to attempt to determine the period of greatest parasitic activity after spore germination, and to produce the toxic principle by growing the fungus for this period only. It is realized that some cases of parasitic action are too complex to allow the accurate determination of this period, but an approximate value may be obtained.

<sup>4</sup> The term "lethal principle" has been adopted because the toxic substance present in the filtrate of *Trichoderma* cultures actually kills the *Rhizoctonia*, while acetone extracts from mats of staling cultures of *Trichoderma* seem to have only an inhibiting effect. The macerating principle that causes the *Rhizoctonia* hyphae to split in later stages of the parasitic action has been found only in the filtrate of staling cultures of *Trichoderma*. \*

### Methods of Obtaining and Testing the Lethal Principle

Under the conditions of the present work, the period of greatest parasitic activity is 2 days after germination of the *Trichoderma* spores. This is shown by the fact that *Rhizoctonia* hyphae, when added to a freshly poured agar suspension of *Trichoderma* conidia, are most vigorously attacked at this time. In obtaining the lethal principle the *Trichoderma* is grown, therefore, for 2 days in 15 cc. of a liquid medium in Petri dishes. This method thus furnishes a concentrated filtrate from a fungus mat of rather uniform development.

An attempt has been made to develop uniform procedures of producing and testing the lethal principle. All the cultures were incubated at 24° C. All the operations were carried out as aseptically as possible. The *Trichoderma* was grown for 8 to 10 days on slants of glucose-potato agar. A heavy spore suspension was made from these slants and freed of hyphae and débris by filtration through a cone of No. 2 Whatman filter paper placed, prior to sterilizing, under the cotton plug inside a large test tube. About 14 million spores were added to each Petri dish containing 15 cc. of the following medium: 1,000 cc. water, 25 g. glucose, 2 g. bactopectone, 2 g.  $\text{KH}_2\text{PO}_4$ , 1 g.  $\text{MgSO}_4$ , .01 g.  $\text{FeCl}_3$ . After 2 days' growth the resulting mycelial mats of *Trichoderma* were removed and the filtrate from a number of dishes was poured through a double layer of fine filter paper (No. 42). The composite sample was then divided into 10 cc. aliquot portions in test tubes. The few *Trichoderma* spores that occasionally escaped through the filter had no effect on the *Rhizoctonia* during the period of the experiments. The filtrate from cultures of 2 days' growth usually had the pH 4.5. The hydrogen-ion concentrations were determined by the quinhydrone electrode. The colorimetric method (6) was used for preliminary tests when quick work was imperative and also for pH values above 8.0.

Equivalent amounts of *Rhizoctonia* hyphae, approximately uniform in age and development, were obtained as follows: From the advancing margin of Petri dish cultures on glucose-potato agar, disks of 3 mm. diameter were cut and placed at equal distances in Petri dishes containing 10 cc. of water agar. The scant growth of *Rhizoctonia* formed within 3 days allowed good microscopical observation. From these cultures, disks 9 mm. in diameter were mounted on glass slides in paraffin cells used as a substitute for van Tieghem cells (41).

One-half cc. of the liquid to be tested was placed on each of 4 disks. The criterion of the lethal effect was the loss of the homogeneous appearance of the protoplasm and of the vacuolate structure of the *Rhizoctonia* hyphae. They appeared either empty or as if filled with a granular material (Fig. 1).

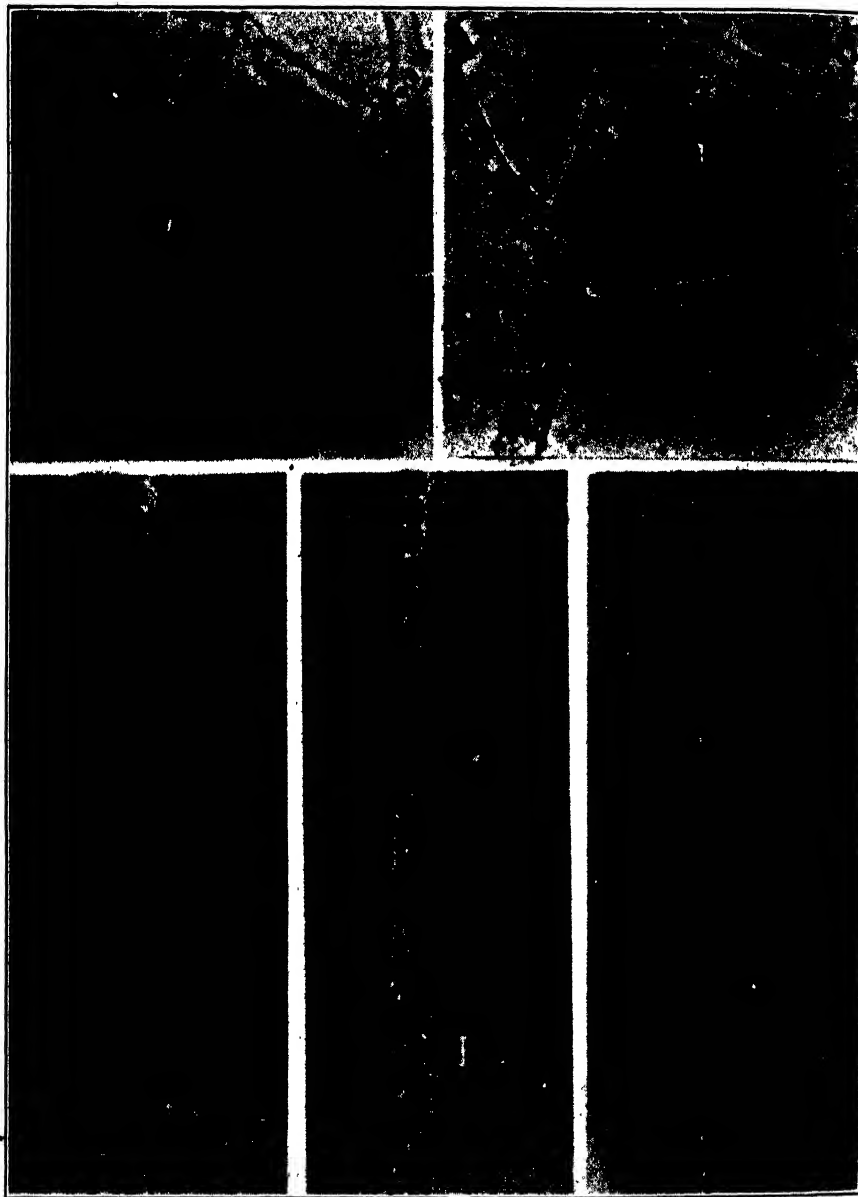


FIG. 1. Effect of the lethal principle on *Rhizoctonia* hyphae. A and C. Live hyphae, 5 minutes after mounting in undiluted filtrate of *Trichoderma* cultures. B and E. The same hyphae dead after one hour. D. Intermediate stage of hypha in C. C-E stained with neutral red.  $\times 660$ .

The measure used here for evaluating the magnitude of the lethal effect of a given filtrate is called its lethal index. It is represented by the reciprocal of the lowest concentration at which all the hyphae of the *Rhizoctonia* are killed within 10 hours. Usually no further lethal effect is observed after this period. For example, if all the *Rhizoctonia* hyphae are killed within 10 hours by a maximum dilution of 1 in 40, the filtrate is considered to have the lethal index 40; if killed by a maximum dilution of 1 in 80, the lethal index is 80. If only a part of the *Rhizoctonia* hyphae is killed by the dilution 1 in 80, the lethal index is between 40 and 80, and is determined according to the percentage of *Rhizoctonia* hyphae killed. Under such an approximate system of computation, allowance for a considerable numerical variation must be made, especially at high dilutions. The amount of variation actually obtained seems to be not too excessive, in view of the difficulties in obtaining uniform procedures of production, test, and evaluation.

For the dilution series 1/10 molecular potassium phosphate buffers of the desired pH were used. The dilutions of the filtrate that were not sufficiently concentrated to be toxic and the buffers served as checks.

#### Production of the Lethal Principle

If the *Trichoderma* is started simultaneously in a large number of Petri dishes and the combined filtrate of 3 of these dishes is tested for its lethal index at frequent intervals, a curve similar to that shown in figure 2 will

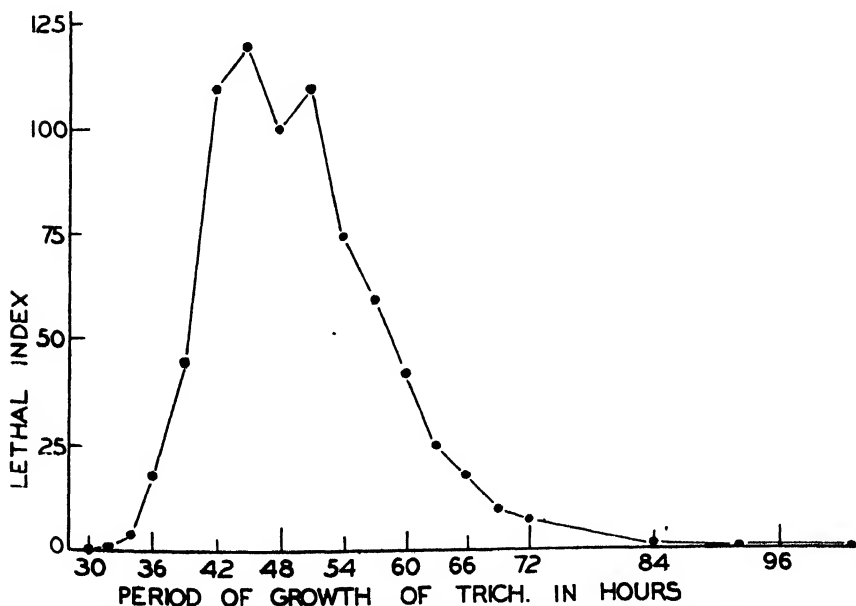


FIG. 2. Lethal indices obtained during the life cycle of *Trichoderma*.

be obtained. The data of 2 experiments of this type are given in table 1, the filtrate being tested at the original pH and at pH 2.5, to which it is adjusted.

TABLE 1.—*Lethal index of the combined filtrate of 3 Petri-dish cultures of Trichoderma lignorum at different periods of growth*

Period of growth	Lethal index			
	Experiment 1		Experiment 2	
	pH 4.5	pH 2.5	pH 4.5	pH 2.5
<i>hours</i>				
30	0	0.5	0	0
32			1	2
33	7	10		
34			4	8
36	25	38	18	30
39	40	60	40	75
42	75	140	110	180
45	100	160	120	220
48	90	150	100	170
51			110	200
54	50	80	75	130
57	35	70	60	120
60	28	65	42	75
63	18	35	25	37
66	7	17	18	28
69			10	15
72			8	
75	3	6		
80	1	2		
84			1	2
92	0	1	0.5	1
104		1		1
132		1		1

Figure 2 may be considered to give an approximate picture of the variation in quantities of the lethal principle produced at the different points of the life cycle of the fungus. There was a sudden rise in production during the second half of the second day, the maximum was near the end of this day, and after a rapid decrease during the third day the rate of production diminished slowly. Abundant sporulation occurred toward the end of this day. It should be emphasized also that during the third day of the growth of *Trichoderma*, the fungus mats will greatly increase in weight, indicating that autolytic processes have not yet set in. However, the rate of production of the lethal principle at this time has already greatly decreased. All attempts to obtain substances lethal to *Rhizoctonia* from the dried,

ground mycelial mats of *Trichoderma* have been unsuccessful. Thus, it appears that the formation of the lethal principle has no connection with autolytic processes. It is not impossible that the lethal principle is an intermediary product of metabolism that may be reutilized by the *Trichoderma* itself.

No lethal effect on the *Rhizoctonia* has been noted after more than 4 days when tested at pH 4.5. But, if the filtrate was adjusted to pH 2.5, lethal action was obtained up to the seventh day. Likewise, filtrates that become effective on *Rhizoctonia* only at pH values lower than 4.0, have been obtained from staling cultures of *Trichoderma* grown for several weeks on Richards' medium in Erlenmeyer flasks. This fact illustrates the inadequacy of this customary method for securing information on toxic substances if they are unstable. The period of maximum production under the conditions of these tests is around 48 hours after sowing the *Trichoderma*. At this time, which closely corresponds to the highest parasitic activity in the life cycle of the fungus, the lethal principle has been secured for use in the experiments described in the paragraphs that follow.

#### DETERIORATION OF THE LETHAL PRINCIPLE AND THE RÔLE OF pH IN ITS EFFECTIVENESS

##### Aerobic Deterioration

A characteristic property of the lethal principle is its rapid decomposition, which, under aerobic conditions, is retarded by low pH values and hastened by high pH values.

The data in table 2 have been obtained by adjusting 10 cc. aliquot portions of the filtrate to different pH values immediately after filtration, storing them at 24° C., and testing the lethal effect of these samples at frequent intervals. At pH 7.5 and 6.0, the lethal effect decreased within only 1½ hours to about  $\frac{1}{10}$  of the original value, and disappeared completely after 3 to 6 hours. At pH 4.5 and 2.5, however, periods 5 to 10 times longer were required to bring about the same changes.

During the time of testing the filtrates on the slides with *Rhizoctonia*, more of the lethal effect is lost at high pH values than at lower ones. The data in table 2 are, therefore, not directly comparable with one another. Another experiment has been conducted to make such a comparison possible. It is identical with those of table 2, except that all the portions of the filtrate stored at 3 different pH values are tested at one pH value, pH 4.5. The curves of figure 3 are thought to indicate the course of decomposition of the lethal principle during one day, as affected by the different pH values at which the decomposition takes place. These curves are in general agreement with the results of table 2, and are corroborated by the following experiments.

TABLE 2.—*Aerobic deterioration of the lethal principle of Trichoderma lignorum at 24° C. Lethal indices obtained at the pH of storing*

Period of storage	Exp. 1		Exp. 2		Exp. 3		Exp. 4			Exp. 5			Exp. 6		
	pH		pH		pH		pH			pH			pH		
	4.5 <sup>a</sup>	2.5	4.5	2.5	4.5	2.5	6.0	4.5	2.5	7.5	6.0	4.5	7.5	6.0	4.5
hrs.															
0	90	140					45	130	240	25	60	120	19	37	110
1	.....	..	110	200	65	100	...	..	..	..	...	..	..	...	..
1.5	.....	..	..	..	..	..	4	..	..	2	4	75	0.5	3	60
3	35	70	40	120	30	75	2	65	160	1	2	55	0	1.5	40
4.5	..	..	..	..	..	..	..	..	..	0	1	28	..	1	32
6	18	40	13	75	6	32	0	35	85	..	0	18	..	0.5	25
7.5	..	..	..	..	..	..	..	..	..	..	..	10	..	0	14
9	10	20	4	37	2	14	..	8	38	..	..	..	..	..	..
12	4	20	2	37	0.5	7	..	1	18	..	..	..	..	..	..
15	1	7	..	..	..	..	..	0	8	..	..	..	..	..	..
24	0	2	0.5	8	0	2	..	..	2	..	..	..	..	..	..
48	..	..	0	4	..	1	..	..	1	..	..	..	..	..	..
72	..	..	..	1	..	0.5	..	..	0.5	..	..	..	..	..	..
96	..	..	..	0	..	0	..	..	0	..	..	..	..	..	..

<sup>a</sup> The figures 7.5, 6.0, 4.5, and 2.5 of this line indicate the pH of storing and testing of the filtrate.



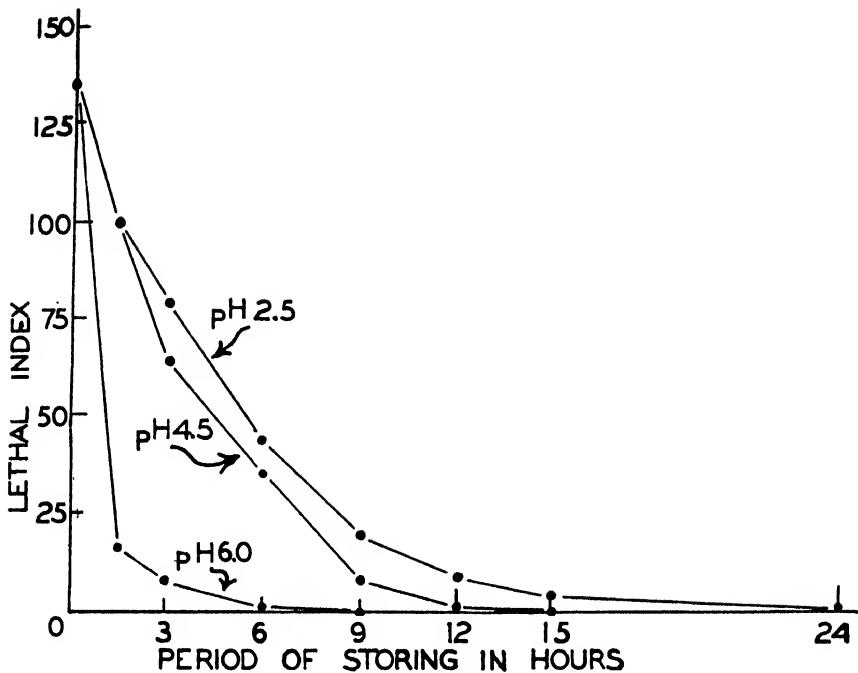


FIG. 3. Influence of pH on the deterioration of the lethal principle. Filtrates kept aerobically at 24° C.

The portions of a filtrate stored at different pH values show the greatest discrepancies as to their lethal indices after certain periods. Of these, 3 hours' storing has been chosen to compare filtrates kept at pH 6.0 and 4.5, and 12 hours' storing selected for contrasting samples stored at pH 4.5 and 2.5. Each aliquot portion was tested at the pH at which it had been stored,

TABLE 3.—*Aerobic deterioration of the lethal principle of Trichoderma lignorum at 24° C., as influenced by the pH of the filtrate*

No. of experiment	Test pH	Lethal index			Test pH	Lethal index		
		Before storing	Stored 3 hrs. at			Before storing	Stored 12 hrs. at	
			pH 4.5	pH 6.0			pH 4.5	pH 2.5
1	4.5	140	35	5				
	6.0	38	9	1.5				
2	4.5	75	30	4	4.5	75	1.5	10
	6.0	32	7	1	2.5	120	3	16
3	4.5	150	50	8	4.5	150	2.5	15
	6.0	60	14	2	2.5	240	4	28

as well as adjusted to the pH of the portion with which it was to be compared. The figures in table 3 indicate that at the chosen periods, the amounts of lethal principle were 5 to 7 times larger in the portions stored at the lower pH values as compared with those of the higher pH values.

There appears to exist a constant numerical relationship between the lethal indices obtained by adjusting one and the same filtrate to different pH values. In table 3 this relationship appears in the pairs of figures placed above one another. If tested at pH 6.0 the lethal index of any given sample is  $\frac{1}{3}$  to  $\frac{1}{4}$  of the lethal index obtained from the same sample at pH 4.5; if tested at pH 2.5, the lethal index is 1.5 to 2 times that of pH 4.5. The same relationship may be noted in tables 1, 5, and 6. This suggests that the chemical nature of the lethal principle is not influenced by the conditions under which decomposition takes place.

#### Anaerobic vs. Aerobic Deterioration of the Lethal Principle

The decomposition of the lethal principle is retarded by low temperatures and by excluding the oxygen of the air. If stored 24 hours at 24° C. at pH 4.5 and tested at the same pH, the filtrate loses its lethal effect completely. In contrast, the lethal indices of filtrates stored at 2° C. or anaerobically at 24° C. will still amount to about one-third of the original

TABLE 4.—*Aerobic vs. anaerobic deterioration of the lethal principle of Trichoderma lignorum. Lethal index of filtrates stored and tested at pH 4.5*

Period of storage	Experiment 1		Experiment 2		Experiment 3
	Aerobic at 2° C.	Anaerobic at 24° C.	Aerobic at 2° C.	Anaerobic at 24° C.	Anaerobic at 24° C.
<i>days</i>					
0	110	110	65	65	130
$\frac{1}{2}$	75	72	37	30	..
1	40	38	19	25	..
2	19	28	17	25	40
3	9	20	6	27	..
4	3	16	..	..	..
5	2	18	2	18	..
6	1	18	..	..	..
7	1	13	0.5	..	..
8	0.5	17	..	..	..
9	..	..	0	..	..
10	0	..	..	15	..
14	..	10	..	..	..
18	..	..	..	..	18
28	..	3	..	..	..
42	..	..	..	15	..
77	..	..	..	..	17

index (table 4). Samples in test tubes were kept anaerobically by placing them in tightly stoppered quart milk bottles from which the oxygen had been absorbed by the addition of 10 g. pyragallol and 100 cc. of 10 per cent KOH. While at 2° C., the lethal effect gradually disappeared within 10 days, it seemed to remain stationary for a long time under anaerobic conditions at a level represented by a lethal index of about 18 at pH 4.5. The longest period thus far recorded is 11 weeks. The apparent losses in one of the experiments presented in table 4 may be due to the repeated opening of the bottles.

Another feature of anaerobic decomposition is that it does not seem to be influenced by the pH of the filtrate if stored at 24° C. In table 5 each sample was tested as in table 3; that is, at the pH of storing as well as at the pH of the samples with which it was to be compared. The lethal indices of samples stored at different pH values, but tested at the same pH, were placed side by side. With anaerobic storing at 24° C., these pairs of lethal indices agree well within the limits of probable variations. With aerobic storing at 2° C., the lethal indices differ greatly according to the pH value at which the material is stored; *e.g.*, when stored at 2° C. at pH 4.5 for half a day, the amount of lethal principle retained is 20–30 times larger than when kept at pH 6.0 under the same conditions. The influence of pH on the anaerobic deterioration of the lethal principle at 2° C. cannot be explained completely with the data at hand. The differences in losses at dif-

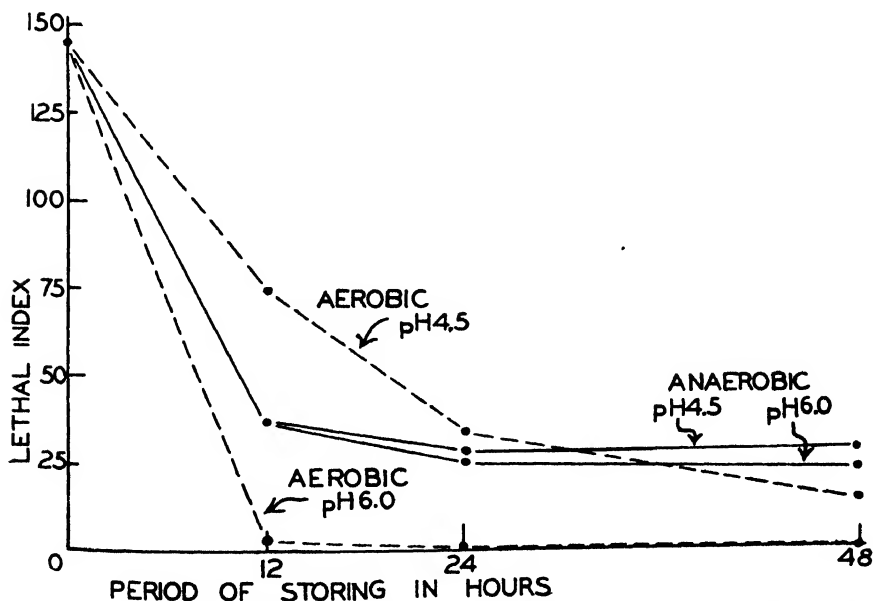


FIG. 4. Influence of pH on the deterioration of the lethal principle. Filtrates kept aerobically at 2° C. and anaerobically at 24° C.



ferent pH values are very much smaller, however, than with aerobic storing at 2° C. They tend to disappear with prolonged storing, thus approaching the effect of anaerobic storing at 24° C.

In figure 4, anaerobic and aerobic storing at pH 4.5 and 6.0 over a period of 2 days are contrasted as to their effect on the lethal action. With aerobic storing at 2° C., the relation of the two curves is similar to figure 3. The curves of anaerobic storing at 24° C., however, are almost identical, indicating that the chemical process of decomposition is different, according to whether it is effected with or without the oxygen of the air.

#### Inactivation by Boiling

In many preliminary experiments the filtrate has been found to retain a small part of its lethal effect if boiled for one or more hours. Even 4 hours at 120° C. has not destroyed it completely.

The data of table 6 were obtained by adjusting aliquot amounts of the filtrate to different pH values and keeping them for 15 min. in a water

TABLE 6.—*Partial inactivation of the lethal principle of Trichoderma lignorum by boiling 15 min. at different pH values*

Number of experiments	Test pH	Lethal index			
		Before boiling	After boiling at pH		
			6.0	4.5	2.5
1	4.5	120	2.0	3.0	2.5
2	4.5	110	1.0	1.5	2.5
3	6.0	35	0.0	0.0	.....
	4.5	100	...	2.0	3.0
	2.5	180	..	4.0	5.0

bath at 99° C. The decomposition of the lethal principle is very rapid, but seems to be rather independent of the pH at which the filtrate is boiled. It is possible that this is, chemically, a type of destruction similar to that occurring under anaerobic conditions. Since the lethal principle does not seem to be a mixture of substances, it may be concluded from this experiment that it is not of enzymatic nature.

#### The Influence of pH on the Effectiveness of the Lethal Principle

Under normal environmental conditions the deterioration of the lethal principle proceeds faster the more alkaline the filtrate is made. Aliquot samples of filtrates were adjusted to a wide range of hydrogen-ion concentrations and their effect tested as simultaneously as possible (Table 7). The procedure involved delayed the testing of the whole series for at least 1½ to 1½ hours after filtration. After this time, no effect was obtained at

TABLE 7.—*The influence of pH on the effectiveness of the lethal principle of Trichoderma lignorum. Mounted 1–1½ hrs. after filtration and adjusting pH values*

pH	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5 <sup>a</sup>
2.5	150	.	140	150	10
3.0	.....	.	120	110	8
3.5	110	..	70	95	..
4.0	.....	..	72	110	6
4.4	... ..	..	65	80	5
4.8	80	60	65	70	..
5.1	... ..	30	55	50	5
5.4	..	17	35	26	..
5.7	... ..	10	20	15	3
6.0	15	6	11	12	2
6.3	... ..	3	6	6	... ..
6.6	... ..	2	3	2	..
6.9	..	1	2	1	1
7.2	3	0.5	2	1	..
7.5	.....	0	1	1	0
7.8	.....	..	1	0.5	..
8.0	0	..	0.5	0	0
8.5	..	..	0	0	... ..
9.0	... ..	..	0	0	... ..
10.0	... ..	... ..	0	0	..

<sup>a</sup> Filtrate from *Trichoderma* cultures 3 days old.

pH 8.5. If this test was made, however, immediately after filtering and adjusting the pH, decided lethal effects were found up to pH 9.8. Nevertheless, the method permits one to visualize the effect of pH on the lethal principle over a wide range of pH values, though under an arbitrary set of conditions (Fig. 5). The curve showing the relationship of pH to lethal index decreases most rapidly between pH 4.8 and 6.3 after which it approaches the zero line more slowly.

#### INTERACTION AND GROWTH OF TRICHODERMA LIGNORUM AND RHIZOCTONIA SOLANI, AS AFFECTED BY THE pH OF THE MEDIUM

##### Methods

Methods for the study of the growth of fungi on liquid media maintained at fairly constant hydrogen-ion concentrations have been reported (24, 35). Since liquid media are not suitable for the microscopic observation of parasitism, most of the present work has been done with strongly buffered agar media of a low nutrient content and adjusted to a series

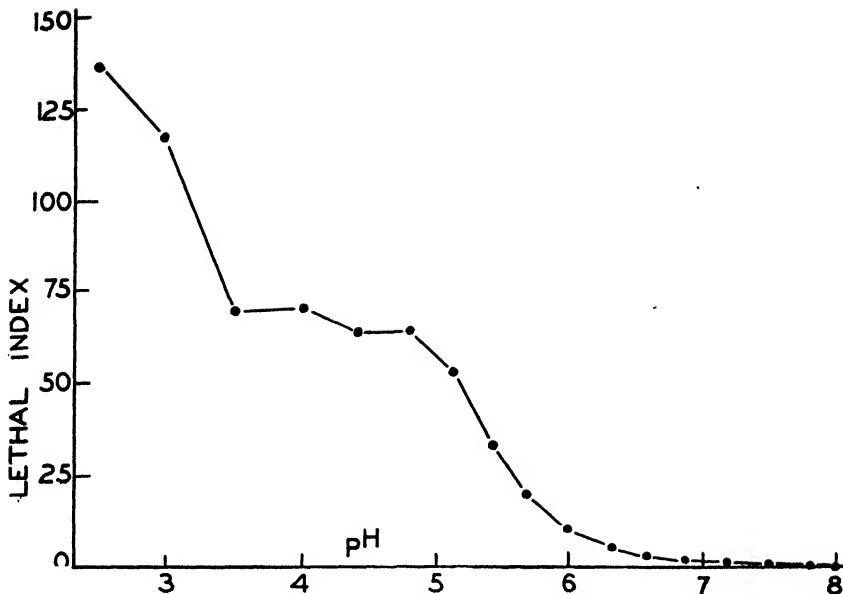


FIG. 5. Lethal effect obtained after adjusting a filtrate to a series of pH values.

of pH values having 0.5 pH intervals. The rather scant growth of the fungi does not tend to change the pH much, and allows good observation of their interaction.

All cultures of *Trichoderma lignorum* thus far isolated from the roots of plants, from decaying lemons, and from the soil have been found to attack the *Rhizoctonia*. They have been tentatively placed in 3 groups: The cultures of the *o* group have a characteristic cocoanut-like odor and readily cause decay of lemons when inoculated into wounds. The *p* and *q* groups possess neither of these properties. The *p* group differs from the other 2 groups by the formation of a yellow pigment under certain conditions. The *q* group appears to be distinguished from the *p* group only by the absence of the yellow pigment. Of the cultures used in this work  $O_1$  and  $O_2$  belong to the *o* group.  $O_1$  is the stock culture of the Division of Plant Pathology at Riverside.  $O_2$  is a recent isolation from lemons. The other cultures belong to the *p* group.  $P_1$  has been used in previous work (40).  $P_0$  and  $P_2$  are single-spore cultures derived from  $P_1$ .

$P_0$  had lost a great deal of its parasitic activity, due apparently to repeated transfers of the vegetative mycelium. A single-spore culture of  $P_3$ , isolated by L. J. Klotz, from the gummosis lesion of an orange tree, has been used for the investigation of the lethal principle reported above.

In preliminary tests with  $P_1$ , Czapek's agar has been used, but with the nutrients and salts in one-half strength. All the other experiments

have been made with media strongly buffered with one-fifth molecular potassium phosphate. The buffer solution has been autoclaved separately and mixed with the tubes of agar just before pouring. In addition to the buffer salts, the media contain in 1,000 cc. water 20 g. agar, 2 g. glucose, 0.2 g.  $\text{MgSO}_4$ , a trace of  $\text{FeCl}_3$ , and 0.2 g.  $\text{KNO}_3$ . In the experiments with  $\text{O}_2$  and  $\text{P}_3$ , 0.2 g. bacto peptone has served as a nitrogen source. The pH is tested in separate tubes by the quinhydrone electrode and, above pH 8.0, colorimetrically. Besides, 5 drops of previously sterilized indicator solutions are added to 1 of the 3 Petri dishes of each set in order to follow the changes of pH during growth. These indicators have not been found disturbing the growth of the fungi.

If only the influence of pH on the growth rates of fast-growing fungi is to be determined, weakly buffered media seem to give results similar to those of strongly buffered ones. The pH changes little in the advancing zone of the colonies, except at very high pH values. This would agree with the results of Fawcett (13) that the growth of certain fungi in solid media does not follow an autocatalytic curve, but continues unchanged because the tips of the advancing hyphae are always growing in media with the original pH. In the zone behind the advancing margin of the colonies, however, considerable changes of pH take place in weakly buffered media, tending toward a reaction between pH 6.0 and 7.0 in the work reported here. The buffered media maintain a nearly constant pH at acid and neutral reactions. The very alkaline media change about as follows: from an original pH 8.0 to 7.6, from pH 9.0 to 8.4, and from pH 10.0 to 8.8.

Most of the data of tables 8 and 9 are based on the average of 3 Petri dishes. The dishes were inoculated with disks of 3 mm. diameter from the advancing margin of fungus colonies on glucose-potato agar. In mixed cultures, the agar disks were placed 3 cm. from one another. The temperature of incubation was maintained at 24° C. in all experiments except that with nonbuffered media, where the temperature rose to 27° C. at times during the summer.

#### Effect of pH on the Growth of *Trichoderma* and *Rhizoctonia*

The data of table 8 have been averaged from measurements in pure cultures that served as checks for the mixed-culture experiments. They also are thought to indicate the differences in relative chances of development of the 2 fungi caused by variations in the pH of soils. The influence of reaction upon the growth of many *Rhizoctonia* strains has been investigated by others (16, 25, 29, 32).

The *Rhizoctonia* grows well over the whole range of pH values occurring in natural soils. Even at pH 3.0, a thin hyphal web is formed. The optimum of the *Citrus* strain seems to extend from about pH 6.0 to pH 9.0



TABLE 8.—*The influence of pH on the growth of Rhizoctonia solani and Trichoderma lignorum. Diameters of colonies in mm. after 42 hrs. growth at 24° C.*

pH	Trichoderma <i>p</i> group				Trichoderma <i>o</i> group		Rhizoctonia		
	P <sub>1</sub> <sup>a</sup>	P <sub>0</sub> <sup>b</sup>	P <sub>2</sub> <sup>b</sup>	P <sub>3</sub> <sup>c</sup>	O <sub>1</sub> <sup>b</sup>	O <sub>2</sub> <sup>c</sup>	a	b	c
1.7	0	0	0	6	0	7	0	0	0
2.0	8	10	8	7	15	23	0	0	0
2.5	22	24	28	38	28	39	0	0	7
3.0	45	31	43	57	35	49	25	27	34
3.5	61	46	49	65	37	56	58	48	52
4.0	70	53	54	65	40	60	67	58	55
4.5	73	49	57	63	43	60	71	60	55
5.0	72	40	60	61	41	60	78	62	55
5.5	70	42	57	61	42	56	79	65	57
6.0	69	43	54	57	42	54	82	67	61
6.5	66	37	47	43	37	38	84	72	64
7.0	64	33	38	37	29	25	85	76	64
7.5	51	28	27	27	22	15	88	79	66
8.0	44	14	13	14	12	5	89	72	65
8.5	41	13	10	13	10	5	90	70	65
9.0	32	11	9	11	0	4	92	69	63
9.5	20	0	0		0		90		
10.0				0		2			63

<sup>a</sup> Nonbuffered modified Czapek's medium.<sup>b</sup> Buffered nitrate medium.<sup>c</sup> Buffered peptone medium.

and perhaps higher (Table 8). While the *Trichoderma* has a slight advantage at acid reactions, its growth rate decreases rapidly on the alkaline side of the range, the optimum range being about pH 4.0 to pH 6.0. This gives a great advantage to the *Rhizoctonia* in alkaline media (Fig. 6).

The difference seems to be large enough to rule out the possible objection that the effect of pH on the growth of the 2 fungi greatly influenced the nutritional and physical nature of the medium (25, 38).

There are slight differences between the growth rates of the *Trichoderma* cultures, especially the relatively better growth of the *o* group in very acid and of the *p* group in very alkaline media. However, the limited amount of data does not yet permit one to generalize these results. The good growth of *Trichoderma* in alkaline media of the nonbuffered series, as compared with the buffered ones, is due to a rapid change towards the neutral point in this first series.

#### Effect of pH on the Parasitic Action

A summary of data concerning the parasitic action of *Trichoderma* on *Rhizoctonia* as influenced by the pH in a series of buffered media is presented in table 9.

TABLE 9.—The influence of pH on the interaction of *Trichoderma* and *Rhizoctonia*

pH	Trichoderma cultures of the p group (pigmented)						Trichoderma cultures of the o group (odor)								
	P <sub>1</sub> <sup>a</sup>			P <sub>2</sub>			P <sub>1</sub>			O <sub>1</sub>			O <sub>2</sub>		
	Rhizoctonia hyphae						Rhizoctonia hyphae								
	Dead <sup>b</sup>	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive			
2.5	All sfc	Most sme	All	None	All	None	All	All	None	All	None				
3.0	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "				
3.5	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "				
4.0	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "				
4.5	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "				
5.0	" "	All sm	" "	" "	" "	" "	" "	" "	" "	" "	" "				
5.5	‡ sf	" "	Most sf	‡ sm	All sf	Few sm	" "	" "	" "	" "	" "				
6.0	Few sf	" "	" "	Many sm	" "	Many sm	" "	" "	" "	" "	" "				
6.5	" "	" "	" "	" "	‡ sf	All sm	" "	" "	" "	" "	" "				
7.0	None	Some ivc	Few sf	Some iv	Few sf	Few iv	" "	" "	" "	" "	" "				
7.5	" "	" "	" "	" "	" "	" "	Many sf	" "	" "	Most sf	" "				
8.0 <sup>d</sup>	" "	" "	None	" "	None	" "	Some iv	Few iv	Some iv	Some iv	" "				
8.5	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "				
9.0	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "				

<sup>a</sup> *Trichoderma lignorum* cultures of o group and p group used for attack on *Rhizoctonia*.<sup>b</sup> State of the *Rhizoctonia* hyphae in the attacked parts of the colonies 5-8 days after inoculation.<sup>c</sup> Abbreviations concerning *Rhizoctonia* hyphae: sf = on the surface of the medium; sm = submerged in the medium; iv = invading the *Trichoderma* colony.<sup>d</sup> Above pH 7.5 there is very little intermingling of *Rhizoctonia* and *Trichoderma* colonies.

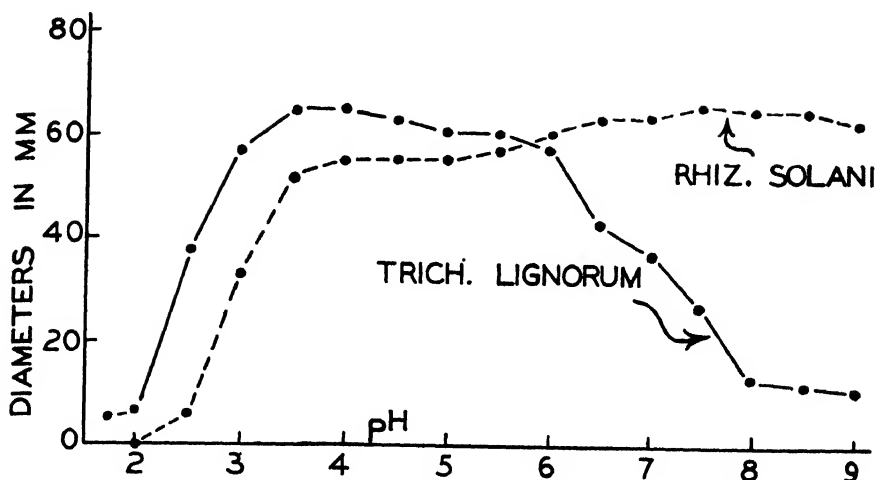


FIG. 6. Diameters of colonies of *Rhizoctonia solani* and *Trichoderma lignorum* grown at 24° C. on a medium containing glucose and peptone for 42 hours at different pH values.

The cultures of the *p* strain showed a rapid decline of parasitic activity at pH values higher than 5.5, although the rate of growth in this range was as rapid as in the range of greatest parasitism. Around pH 5.5 the lethal principle of *P*<sub>3</sub> had also been found to show the greatest increase in deterioration and, subsequently, the greatest loss in effectiveness (Fig. 5). At the marginal pH values the *Rhizoctonia* hyphae submerged in the medium had a better chance to survive, because the lethal principle may deteriorate before it can diffuse to them. With the host hyphae at the surface of the agar this loss was decreased, the *Trichoderma* providing for a large production of the lethal principle and its rapid passage onto the *Rhizoctonia* hyphae by coiling around them or increasing the touching surface in every conceivable way. At the higher pH values coiling occurred occasionally but the *Rhizoctonia* hyphae were not killed. This indicates that the decomposition of the lethal principle was so rapid that it had no effect.

At pH values lower than 5.5 no living hyphae of *Rhizoctonia* were found after the attack of *Trichoderma* cultures *P*<sub>2</sub> and *P*<sub>3</sub>. The same result had been obtained in the nonbuffered series (not included in table 9) at an initial pH 3.0 only, the reaction of all the other media having changed sufficiently toward neutrality to allow the survival of at least a part of the *Rhizoctonia* colony. The culture *P*<sub>0</sub>, which appeared to have lost its parasitic ability on ordinary media, was found to produce an amount of lethal principle just sufficient to kill the surface hyphae of *Rhizoctonia* colonies up to pH 5.0, the submerged hyphae, however, remaining alive (Table 9).

Unfortunately, the lethal index of this culture was not determined because it had been discarded before the standard methods had been developed.

The 2 cultures of the *o* group killed the *Rhizoctonia* also in media around the neutral point (Table 9). This would explain an observation often made with ordinary culture media of a pH 5.5 and higher, in which the *Trichoderma* cultures of the *o* group destroy the *Rhizoctonia* colonies completely, while with the *p* group, sclerotia and living hyphae are found after the *Trichoderma* attack. Before drawing general conclusions, however, more cultures of both groups have to be examined as to the effect of pH on their action. The lethal principle of the *o* group has not yet been studied in detail. Compared with those of the *p* and *q* groups, it has been found surprisingly weak in preliminary tests made under the standard conditions for  $P_3$ .

A basidiomycete isolated by D. E. Bliss from the roots of diseased date palms was subjected to the attack of *Trichoderma* over a range of pH values. The results were similar to those obtained with the *Rhizoctonia* as a host.

#### MISCELLANEOUS EXPERIMENTS

Cultures of *Trichoderma Koningi* Oud. and *T. album* Preuss were secured through the courtesy of the Centralbureau voor Schimmelcultures, Baarn. They were found to attack the *Rhizoctonia* in the same way as *T. lignorum*.

Attempts towards determining the nature of the lethal principle of the pigmented *Trichoderma* culture  $P_3$  have thus far led to the following results: If the filtrate was passed through a Chamberland B. filter, or concentrated under reduced pressure to a syrup, a part of the lethal effect was retained. A toxic effect on *Rhizoctonia* was obtained from the residue of ether extracts of the *Trichoderma* filtrate. Most of the lethal principle seemed to be adsorbed by charcoal when a sterile suspension of it was added to the media together with the *Trichoderma* spores. Likewise, by adding a suspension of animal charcoal to mixed cultures of *Trichoderma* and *Rhizoctonia*, it was possible to suppress the parasitic action of *Trichoderma* very greatly, without apparently influencing its growth. This was probably not due to an effect on the production of the lethal substance, but rather to its adsorption. This phenomenon may be of importance in connection with attempts to purify the lethal principle and in an understanding of the effect on the parasitic action of soils rich in colloidal material.

Further additions to the host list of *Trichoderma* are: *Armillaria mellea* (Vahl.) Quel., 2 undetermined basidiomycetes, and *Rhizoctonia bataticola* (Taub.) Butler. No resistance against attack of *Trichoderma* has been found in a number of strains of *Rhizoctonia solani* from different host

plants obtained through the courtesy of G. R. Townsend, of Cornell University.

An interesting change of pH was noted in the interaction of *Trichoderma* O<sub>1</sub> with *Rhizoctonia*, when grown on Czapek's agar to which a few drops of brom cresol purple indicator had been added. The dying *Rhizoctonia* hyphae took up the indicator and, having a more acid reaction than the surrounding purple medium, stained yellow. After some time the acid seemed to diffuse from the hyphae into the medium, rendering it yellow (pH 5.4 and lower), while outside the zone of dead *Rhizoctonia* hyphae, the medium remained purple (pH 6.4 and higher).

In a similar way the dying *Rhizoctonia* hyphae seem to accumulate under certain conditions the pigment of *Trichoderma* and again lose it, as previously described and illustrated (40). No other connection between pigment and parasitic action has thus far been observed. Like the lethal principle, most of the pigment is adsorbed by charcoal, and it may be extracted by ether from the filtrate. The ineffective filtrate from old cultures of *Trichoderma* of the *p* group, however, contains very much pigment, and the lethal index of culture filtrates of the nonpigmented *q* group is similar in amount to that of the *p* group. This latter group may be identical with the strain of *Hypocrea rufa* Pers. on the yellow pigment of which Medisch (26) has done a considerable amount of work.

Swarm spores of *Phytophthora citrophthora* also have been killed by concentrations of the lethal principle similar to those effective on *Rhizoctonia*.

#### DISCUSSION

The main purpose of the remarks that follow will be to point out some phases of the problems opened up by the work done. Some of the fundamental aspects of the subject having been recognized, further investigation is necessary to gain a more comprehensive view of the problems.

Brown (5) has sufficiently emphasized the importance of uniform methods in obtaining toxic substances produced by fungi at the time of highest parasitic activity. His view is supported by the work reported here. Many so-called facultative parasitic fungi need apparently to acquire a certain vitality through a period of saprophytic growth before they are capable of parasitic activity. With the *Trichoderma* this transition from the saprophytic to the parasitic habit seems to be dependent on the production of a sufficient amount of lethal principle, which, under the conditions used, took place about 1½ days after germination of the spores. Due to continuous deterioration, the lethal principle of *Trichoderma lignorum* does not accumulate during the growth of the fungus in the culture medium. This makes it possible to secure an approximate curve of the rate of production of the

lethal principle during the life cycle of the fungus (Fig. 2). The true curve of the rate of production would exclude the residual amounts from previous production that have not yet decomposed. The slope of this curve would rise, therefore, somewhat slower and descend more rapidly than is actually shown in figure 2. The possibility that the rapid decline in the curve of production during the third day may be due to a rise in pH, which increases the rate of decomposition of the lethal principle, is excluded by the fact that the pH of the medium did not change to any considerable extent before the fourth day of these experiments.

The nature of the toxic substances produced by parasitic fungi has been found to differ widely. Endoenzymes (5) and exoenzymes (1, 12, 15) have been reported. More or less definite chemical compounds, like nitrites (21, 31), aldehydes (20), or organic acids (17) have been isolated and thought responsible for the toxic effect of the filtrates. Certain organic acids have been found toxic to some fungi at very low concentrations (19). As to the chemical nature of the lethal principle of *Trichoderma lignorum*, no conclusive evidence has been presented. There is, however, one significant property of the lethal principle remaining constant under extremely varying conditions. The ratio between the lethal indices obtained by adjusting the filtrate to certain pH values does not seem to be much affected, whether the lethal principle is secured at different stages of growth of *Trichoderma*, or obtained as a residue from different degrees of decomposition by boiling and by aerobic or anaerobic storage at different temperatures. This should hardly be expected if it were a mixture of different substances. It seems to indicate that the lethal principle is a single definite chemical substance that is readily oxidized and of nonenzymatic nature. It is possible that this substance is a specific product of metabolism of species of *Trichoderma*, such as the substances found specific for certain groups of fungi in the excellent work of Raistrick and his collaborators (30). A group of *Penicillium* species has been found to produce a specific substance, penicillin, which, in very high dilutions, is able to suppress certain bacteria (14). This substance is said to be nonenzymatic and rather unstable.

In the determining effect of pH on the interaction of *Trichoderma* with *Rhizoctonia*, the instability of the lethal principle seems to be the deciding factor, although there is no proof that it is the only one. There is, under a definite set of conditions, a certain pH range at which the parasitic action decreases rapidly, no parasitism being exhibited at higher pH values. Apparently, in this range of pH values, the decomposition of the lethal principle proceeds so fast that the amount left becomes smaller and smaller with increasing pH, until it is not sufficient to kill the *Rhizoctonia*. It is evident that this pH range is not absolutely fixed, but will be influenced to a certain extent by many factors. They may be external, like nutrients,

moisture, temperature, etc.; or they may concern the fungi themselves, such as types, stages of development, and relative amounts of parasitic and host mycelium.

Besides, environmental factors have an indirect influence on the parasitic action, by affecting the growth and development of each of the fungi; e.g., the growth of *Rhizoctonia* has been seen to be at a greater advantage, as compared with *Trichoderma*, the more alkaline the medium becomes. A medium of low moisture content is more unfavorable for the growth of *Trichoderma* than for *Rhizoctonia*. On the other hand, Falck (11) finds that *Trichoderma* species are able to get established in water-logged lumber before the dry-rot fungi, and that the later invasion of the wood by the dry-rot fungi is checked more or less by toxic substances excreted by *Trichoderma*. A similar rôle may be played by *Trichoderma* species in water-logged soils, in subsoil, and certain forest soils, where it is reported to be abundant (7, 37).

Another important problem is the great difference in aggressiveness of different groups of *Trichoderma lignorum* and other species of *Trichoderma*. In this connection, the possible rôle of prolonged artificial culture in decreasing the parasitic ability needs to be considered. A wide field is also opened up in the study of resistance against *Trichoderma* attack by comparing the effect of the lethal principle and of the parasite on the wide range of hosts, which are probably more numerous than has been hitherto reported (3, 27, 40). Such studies also may give a better understanding of some soil-microbiological processes and their relation to the action of plant-pathogenic organisms.

Preliminary experiments on the rôle of the interaction of *Trichoderma* and *Rhizoctonia* in the soil have borne out the results of the work in culture. By merely adding a spore suspension of *Trichoderma lignorum* to a sterilized soil at the time of a heavy inoculation with *Rhizoctonia solani*, Citrus seedlings were better protected from damping off at reactions below pH 5.5 than at reactions higher than pH 5.5. *Trichoderma* culture  $O_2$ , which was found more effective than  $P_3$  in culture, also appeared to be more actively parasitic in soil. These facts explain the variable results of mixed inoculation of soils in former experiments (39, 41). Together with good growth of *Trichoderma* at acid reactions, the influence of low pH values on the interaction with other organisms may contribute to the prevalence of *Trichoderma* species in acid soils (36, 37). Bisby and his associates (3) report an experiment in which the *Trichoderma* has rendered harmless the inoculation of wheat with foot rot fungi in sterilized soil.

*Trichoderma* species are of universal occurrence as soil organisms. They were shown to be extremely active in the decomposition of soil organic matter (37). The above reports indicate that these fungi, under favorable

conditions, may play an additional beneficial rôle in the soil, reducing plant diseases by attacking soil-borne pathogenic fungi.

Recent work on the antagonism between saprophytic soil organisms and plant pathogens (8, 9, 22, 33) is concerned mostly with surveying the effect of a large number of antagonistic organisms towards a few plant-pathogenic fungi. As a supplement, it seems necessary to single out individual antagonistic organisms, and to study their behavior towards one or more plant pathogens, employing in such an investigation accurately controlled environmental conditions.

#### SUMMARY

A lethal principle has been found to be instrumental in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi.

Uniform procedures have been developed for obtaining and testing the lethal principle of the pigmented culture of *Trichoderma lignorum*. In order to compare the lethal effects of various toxic solutions, the numerical expression "lethal index" is employed. The lethal index is represented by the reciprocal of the minimum concentration at which the *Rhizoctonia* hyphae are killed.

The approximate curve of production of the lethal principle during the life cycle of *Trichoderma* indicates that the lethal principle is excreted into the surrounding medium by young hyphae only. There is evidence suggesting that the lethal principle is a single chemical substance. It seems to be neither of enzymatic nature nor a result of autolytic processes.

A characteristic property of the lethal principle is its rapid decomposition. Under aerobic conditions, the rate of deterioration increases with increasing pH. This process is much slower at 2° C. than at room temperature. Anaerobic decomposition of the lethal principle, however, is rather independent of the pH of the medium, and does not seem to go to completion. Boiling of the filtrate greatly weakens the lethal principle, but even prolonged autoclaving does not destroy it completely.

If portions of the filtrate containing the lethal principle are adjusted to a series of pH values, the lethal effect is found to decrease with increasing pH. This is believed to be due to the more rapid decomposition at the higher pH values.

The effect of the lethal principle of *Trichoderma lignorum* and the action of the fungus itself on *Rhizoctonia solani* parallel each other in a number of ways. (1) The maximum amount of lethal principle is produced 2 days after the germination of the *Trichoderma* spores. Likewise, the strongest parasitic action occurs at that time. (2) The effectiveness of the lethal principle has been found to decrease most rapidly in the pH range between pH 4.8 and 6.3. In approximately the same range the pigmented



cultures of *Trichoderma lignorum* gradually lose their ability to parasitize the Rhizoctonia, no attack taking place at alkaline reactions. (3) If an adsorbing agent, such as animal charcoal, is added on sowing the Trichoderma spores, filtrates very poor in lethal principle are obtained, and, in the presence of the Rhizoctonia, the parasitic action of Trichoderma is greatly inhibited.

The group characteristics and the effect of prolonged artificial culture of *Trichoderma lignorum* seem to play a great rôle in the amount of lethal principle produced and in the pH range of parasitic action. Cultures of the fungus that are characterized by a cocoanut-like odor have been found to attack the Rhizoctonia at neutral and alkaline reactions at which no parasitism is exhibited by pigmented cultures.

A comparison of the pH growth curves of *Trichoderma lignorum* and *Rhizoctonia solani* shows that alkaline reactions are favorable to Rhizoctonia but unfavorable to Trichoderma.

Good control of damping off of Citrus seedlings due to *Rhizoctonia solani* has been secured by adding Trichoderma spores to a sufficiently acid, sterilized soil.

*Trichoderma Koningi* and *T. album*, as well as all isolations of *T. lignorum* tested, have been found to attack the Rhizoctonia.

*Trichoderma lignorum* also is capable of parasitizing *Rhizoctonia bataticola* and *Armillaria mellea*.

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# SPECIFIC QUANTITATIVE NEUTRALIZATION OF THE VIRUSES OF TOBACCO MOSAIC, TOBACCO RING SPOT, AND CUCUMBER MOSAIC BY IMMUNE SERA

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Chiefly through the studies of Beale (9, 10) the attention of a number of investigators has recently been directed to an analysis of the possible antigenic capacity of the plant viruses. It has been known for a number of years that the animal viruses are capable, in general, of engendering in inoculated susceptible and nonsusceptible animals the production of specific neutralizing antibodies, and possibly of specific precipitins as well. From the works of Beale and their confirmation and extension by Matsumoto (5), Silberschmidt (11), Gratia (2), and Birkeland (1) we know that the inoculation of rabbits with preparations containing tobacco-mosaic virus results in sera that precipitate such virus preparations at dilutions of 1:100 or higher, that the reaction in question occurs regardless of the host species harboring the virus, so far as has been tested, that the reaction occurs without loss of precipitin titer even though the virus has been subjected to purification by various methods, and that the reaction appears to be specific for the virus used as inoculum. It thus would appear that the antigenic substance involved is either the virus itself, or else some close combination of virus with other substances present in the plant juices.

Up to the present no satisfactory evidence has been advanced that serum immune from a plant virus is capable of neutralizing or inactivating that virus. Beale (9) and Silberschmidt (11) have used the term "neutralization," referring to their studies on the inhibition of tobacco-mosaic virus by serum; but in these works it was not made clear whether this inhibition operates through an effect on the virus (neutralization) or through a lowering of the susceptibility of the host plant. To be sure, the term neutralization is used by animal serologists, although a possible effect on the host might be conceived. Yet the methods of inoculation differ so markedly in the two fields that this practical source of error with the plant viruses may have but slight theoretical importance as regards the animal viruses. Holmes (3) has shown that toxic materials in tobacco sap will decrease tobacco-mosaic infectivity through a deleterious effect on the host. Recently, Stanley (12) pointed out that trypsin likewise reduces the infectivity of tobacco-mosaic virus not by affecting the virus but by an effect on the host plant. Thus it is evident that before the term neutralization may

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be applied to the action of serum when mixed with virus preparations, it is first necessary to determine whether or not the effect observed is actually an effect on the virus involved.

Since neutralization is necessarily an effect, direct or indirect, on the virus itself, not primarily involving the non-virus constituents of virus preparations, it has been felt that an investigation of the ability of plant viruses to induce in animals the production of neutralizing antibodies might add instructive evidence to that obtained from a study of the precipitin reaction. One of the ultimate ends of serological studies with the plant viruses is a conclusion as to the fact and extent of the antigenicity of these viruses; but, since the studies here reported are being continued, it is felt advisable to postpone an analysis of the whole question of plant-virus antigenicity until more evidence is in hand. The present paper is accordingly confined to a report of investigations of the neutralization of certain plant viruses by immune serum.

#### LITERATURE

In 1926 Mulvania (6) first published an account of researches involving the inhibitory effect of rabbit serum on the virus of tobacco mosaic. Mulvania found that the addition to virus preparations of fresh, normal rabbit serum caused a decrease in infectivity of the virus. The infection experiments were performed by inoculating ten tobacco plants with each of the serum-virus mixtures. Two years later Purdy (9), using a similar technique in a more extensive series of experiments, confirmed Mulvania's finding and reported further that serum of rabbits immunized with tobacco-mosaic extract is more effective than normal serum in reducing the percentage of infectivity of serum-virus mixtures. Matsumoto in 1930 (5) again noted the inhibitory effect on tobacco-mosaic virus of antiserum, and in 1933 Silberschmidt (11) confirmed the accounts of these previous investigations in showing that mixtures of virus and antiserum, if plated out on leaves of *Nicotiana glutinosa*, produce fewer local lesions of the tobacco-mosaic disease than do corresponding mixtures of normal serum and virus, although the latter mixtures showed some decrease in infectivity as compared with serum-free controls.

From the works cited one is justified in concluding that the addition of normal rabbit serum to tobacco-mosaic virus causes a decrease in the infectivity of the virus preparation, and that the addition of mosaic-immune serum causes a still further decrease in infectivity. Beyond this one must proceed with some reservation in analyzing these earlier works, since any error due to host effect was not considered, and since, in a large part of the work reported, the method used for determination of virus infectivity (pin-prick inoculations of a few tobacco plants) was relatively insensitive, as

compared with the more efficient method of plating virus on local-lesion hosts.

Preliminary to the present study, the findings of the four investigators mentioned above were confirmed, and it was found that by the use of comparable techniques the inhibiting effect of normal serum and the greater inhibitory effect of antiserum were readily demonstrated.

The question now arises as to whether the relatively weak inhibitory effect of normal serum and the stronger inhibitory effect of immune serum are qualitatively different. The problem resolves itself into a determination of whether the inhibitory effect of serum is due to a decrease in the susceptibility of the host or to an inactivation of the virus. An effect on the host would serve to explain the results thus far recorded, but only a specific, quantitative effect on the virus itself would justify the use of the serological term neutralization.

#### MATERIALS AND METHODS

*Virus stocks.* Viruses of the following types and sources, all propagated in plants of *Nicotiana tabacum* var. Turkish, served as infective material for the experiments reported:

- a. *Tobacco mosaic*: A strain that had been repeatedly purified by single-lesion isolations was obtained from J. H. Jensen.
- b. Several brilliant strains of tobacco mosaic isolated by J. H. Jensen (4) and obtained from him. Of these brilliant strains those particularly employed in the present study were "Jensen 108," a white mottling strain, which causes very severe distortion and stunting, and "Jensen 306," a moderately yellow variant.
- c. A symptomless strain of tobacco mosaic obtained from F. O. Holmes.
- d. *Tobacco ring spot*: An unpurified strain of common green type.
- e. *Cucumber mosaic*: A green strain (Porter's No. 1), and a brilliant yellow strain obtained from W. C. Price and designated by him as No. 6 (8) both of which he had repeatedly purified.

*Virus Preparations.* The following types of virus preparations were successfully used in the experiments to be reported:

- a. Expressed sap of diseased plants either used at once after expression, as was always done with the viruses of cucumber mosaic and tobacco ring spot, or kept frozen until needed (virus of tobacco mosaic and its strains).
- b. Extracts of fresh diseased tissues macerated in two parts of physiological saline solution, cleared, and kept frozen until needed (tobacco mosaic and its strains).
- c. Tobacco-mosaic virus purified by basic and neutral lead acetate precipitations (13) and kept frozen until needed. This type of preparation was used most extensively with tobacco mosaic because of its high infectivity and relative freedom from non-virus materials.

*Host Plants.* Infectivity of virus samples and virus-serum mixtures was determined by counts of the local lesions produced on the primary leaves of the bean, *Phaseolus vulgaris* L. var. Early Golden Cluster, and occasionally, on young leaves of *Nicotiana glutinosa* L., in the case of tobacco mosaic. Cucumber mosaic and tobacco ring spot were similarly titrated on the primary leaves of the cowpea, *Vigna sinensis* (L.) Endl. var. Black Eye, according to techniques described by Price (7, 8).

*Technique of Inoculation of Host Plants.* Inoculation for the propagation of stock virus material was by the pot-label method (4).

Inoculation of beans and cowpeas for infectivity counts was by uniformly rubbing leaves with a piece of folded gauze moistened with the solution to be tested. Rigorous care was taken to prevent accidental infections, high dilutions always being plated out before low dilutions, hands being thoroughly washed between inoculations of any two different solutions, and the inoculated leaves being rinsed a few minutes after inoculation.

*Preparation and Care of Sera.* Rabbits, weighing approximately 1500 gm., were used exclusively for the production of sera. Eight injections at 3- or 4-day intervals were employed, the customary practice being to inject 2 cc. intraperitoneally for the first injection, followed by 7 intraperitoneal injections of 5 cc. each. Intravenous injections of similar amounts of inoculum at similar intervals were employed in preliminary work, but the added labor and danger was not compensated for by any superiority in titer of the intravenous sera. Of 35 animals immunized only 3 were lost, 1 from coccidiosis, 1 from shock following an intravenous injection, and the third apparently from septicæmia.

In preliminary experiments virus solutions for injection were prepared by centrifuging 1:2 saline extracts of fresh virus tissues,—later, and in the greater part of the work here reported, centrifuged expressed saps were employed, the expressed saps giving somewhat more potent immunization than the extracts.

Five to 10 cc. of normal serum was taken from each animal prior to immunization. This serum was frozen and used as control normal serum for the immune serum subsequently obtained from the same animal. Nine or 10 days following the 8th inoculation, after 24 hours without food, the animals were anaesthetized with ether and bled from the carotid artery. The blood was allowed to clot 2 hours at room temperature and over night at a temperature a little above freezing, then the serum was decanted, centrifuged twice, and kept frozen solid until needed. Ordinarily there was little or no sign of deterioration as regards either neutralizing or precipitating power, even in sera that had been several times momentarily melted and then refrozen, although in two or three exceptional cases sudden loss of titer was observed. Sera used 6 months after the original freezing, a

period interrupted several times by partial melting, were usually fully as potent as originally.

*Serum-virus Mixtures.* In preparation of a neutralization experiment a series of dilutions of virus were prepared, the dilutions being in physiological saline, Ringer solution, or M/10  $K_2HPO_4$ . To each sample of each dilution of virus was then added a constant amount of normal or immune serum-dilution, the sera being diluted in the same solvent as the virus. A series of virus dilutions mixed with the serum diluent was used in every case as a control. Wherever possible the immune and normal sera used in any given experiment originated in the same animal. With cucumber-mosaic and tobacco-ring-spot viruses the highest virus dilution practical was 1:100; with tobacco mosaic, infections have been obtained in virus stocks diluted 1:10,000,000, although 1:20,000 and 1:200,000 were the highest dilutions employed in routine work. The sera tested against cucumber mosaic and tobacco ring spot were invariably used at a net dilution of 1:50 (2 normal drops of a  $\frac{1}{2}$  solution being added per 1 cc. of virus dilution). With tobacco mosaic the sera were usually diluted 1:200 (1 cc. of 1:100 serum dilution per 1 cc. virus dilution), although recent work has shown that serum dilutions of 1:1000 or higher are even more satisfactory when it is desired to minimize the inhibitory effect on virus of normal serum, and when relatively potent virus preparations are used.

It has been found advisable to absorb all sera, normal and immune, with 2 parts of healthy tobacco sap, for 2 hours at 37° C., followed by centrifuging and the making of proper allowance for dilution by the healthy tobacco sap. This has proved advisable, since even normal serum produces a slight precipitate with tobacco-mosaic virus preparations, particularly when the serum is in excess, and this precipitate mechanically removes from suspension a certain amount of virus, which tends slightly to distort the results.

Mixtures of tobacco-mosaic virus and absorbed serum were repeatedly shaken during an incubation time of 1-2 hours at room temperature, then plated out directly. With the viruses of cucumber mosaic and tobacco ring spot this time was usually reduced to  $\frac{1}{2}$ -1 hour. Incubation at 37° C. is unnecessary and fails to intensify appreciably the inhibitory effect of anti-serum.

*Care of Apparatus.* All glassware was cleaned with a dichromate- $H_2SO_4$  solution and thoroughly rinsed before using. Further sterilization was found to be unnecessary. The same treatment was applied to the porcelain mortars used in maceration. Hypodermic needles were cleaned in strong boiling sodium bicarbonate solution, then washed and sterilized by boiling. Gauze inoculation pads were prepared from new gauze and sterilized in steam before using.

*Infectivity Counts.* With the cowpea, the number of local lesions pro-



duced per leaf is never too great to be counted with ease. With the bean, the number of lesions per leaf frequently reaches 1600 or more. In such cases it was customary to count accurately all leaves with less than 50 lesions, while leaves bearing more were estimated as to number by comparing with a plaque of dried bean leaves bearing a series of counted numbers of lesions. With tobacco mosaic, it was customary to use 16-18 leaves for each mixture containing virus at less than 1:2000 dilution, and 32-72 leaves for more dilute samples. With cucumber-mosaic and tobacco-ring-spot viruses, 20 leaves were used for dilutions below 1:10 and 30-80 leaves for more dilute samples. Whenever a strong inhibitory effect was anticipated, such as to decrease the count markedly, as with tobacco-mosaic virus and tobacco-mosaic antiserum at virus dilutions below 1:2000, correspondingly greater numbers of leaves were employed. Twelve dozen pots of plants (750 culled leaves of bean, 1000 culled leaves of cowpea) were customarily used for each experiment involving the testing of 3-4 virus-serum mixtures with controls at various dilutions. Each experiment reported has been repeated at least once with confirmatory results. The experiments on occurrence of the neutralization reaction and on its specificity have been repeated many times with no essential departure from the results indicated below.

*Plan of Experimentation.* Since, as has been pointed out above, it was first necessary to determine, if possible, whether the inhibitory effect of normal or immune serum is an effect on host susceptibility or on virus, the following type of experiment was devised for differentiating these two effects. If the serum effect is on the susceptibility of the host, then a given amount of serum would be expected to produce the same percentage of decrease in susceptibility regardless of the amount of virus simultaneously applied; if, on the other hand, the effect of the serum is on the virus itself, then a relationship would be expected to exist between serum and virus such that a given amount of serum would hardly have a detectable inhibitory effect if an excess of virus is present, while as the amount of virus is decreased, the amount of serum being held constant, the relative inhibitory effect would become proportionally greater. From the data obtained from such an experiment, if the percentage of infectivity as compared with the serum-free virus control at each dilution be plotted (ordinate) against degree of virus dilution (abscissa), then one might have a means of differentiating the host-effect from the virus-effect, the former yielding a horizontal line, depressed below the control (100 per cent infectivity) to an extent determined by the constant absolute amount of serum added, the latter giving a curve essentially coincident with the control horizontal where the amount of virus is in excess of the amount of serum, but rapidly curving downward as the amount of virus approaches the amount that can be completely neutralized by the constant amount of serum employed.

Such an experimental arrangement was accordingly attempted, and, preliminary tests having shown its suitability, the same arrangement with such modifications as were necessary in the elucidation of various points, has been made the basic type of experiment in the present investigation.

#### EXPERIMENTATION

##### The Characteristic Effects of Normal Serum and Immune Serum on Virus Infectivity

Normal serum and tobacco-mosaic immune serum, each at net dilutions of 1:200, were respectively mixed in constant amounts with a dilution series of lead acetate purified tobacco-mosaic virus, and the results of infectivity counts were plotted in terms of the infectivity of the serum-free control, as was projected above. The results are illustrated in figure 1, which represents one of many such experiments.

It is seen from the figure that normal serum has an effect of lowering the percentage of infectivity by essentially the same degree throughout the whole of the experimental range of virus dilutions, from  $1/2$  to  $1/20,000$ , and with a variability relatively low for a biological test. The only point that fails to conform well to a parallel position for the two curves is the first, at virus dilution  $1/2$ . The explanation of this single abnormally high point, which is characteristic for the curve wherever tested, is apparent when one considers the effect of dilution of the virus alone. From dilutions  $1/2000$  to  $1/20,000$  the drop in infectivity for the normal curve as given in absolute terms above the curve is approximately proportional to the factor of 10 used in this step of dilution. As we proceed to the left, however, there is less and less proportionality until from  $1/2$  to  $1/20$  the number of lesions is decreased by only a little more than half. This flattening of the dilution curve of tobacco-mosaic virus in the higher concentrations of virus is due to two elements, one of which, as will be seen below, is an effect of the normal constituents of tobacco juice on the host plant, an effect that in this case was at a minimum because a purified virus preparation had been used; while the other element may be termed the saturation of the bean tissues for virus at high concentrations. The mean number of lesions per leaf of the control virus at  $1/2$  was 1220. It would be easy to double the concentration of this virus, yet more than 1600–2000 lesions have never been seen on a bean leaf. Thus at very high concentrations of virus the limit is approached at which the amount of virus can be no longer proportionally registered by the number of lesions appearing. Some lesions at these higher concentrations are doubtless caused by more than one infective unit of virus. Hence the absolute number of lesions approaches a maximum beyond which it cannot pass regardless of the concentration of virus, a limit conditioned by the ability of the bean leaf to register lesions. The

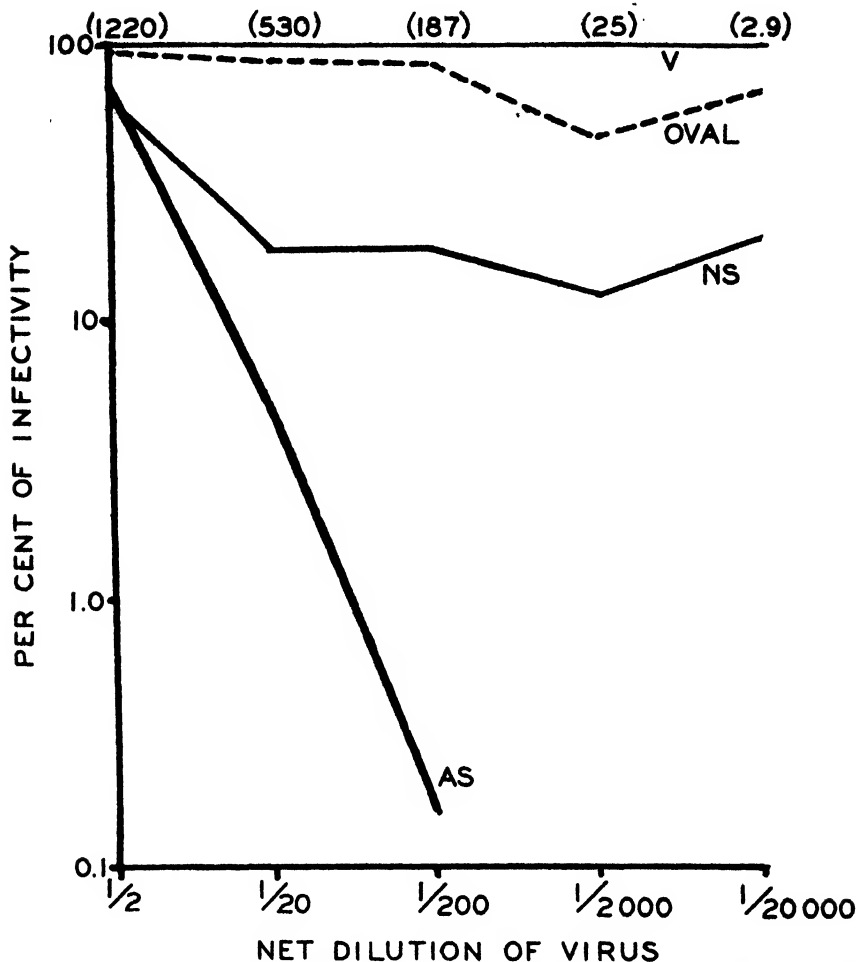


FIG. 1. Percentage of inhibition of tobacco-mosaic virus by normal serum (NS) at 1: 200, by anti-tobacco mosaic serum (AS) at 1: 200, and by ovalbumin (Oval) at 1: 200, all in terms of infectivity of untreated virus control (V). Percentage of inhibition determined by infectivity counts on bean. In this and the graphs to follow, the absolute values for the control curve, V, are given in parenthesis above each point of the dilution scale, and from these absolute values the value of any point on any curve can readily be computed. Further discussion in text.

number 1220 of our experimental virus at 1/2 is not far from that maximum. Meanwhile, the serum-treated samples at the same dilution gave only about 2/3 that number of lesions, *i.e.*, with them the depressing effect due to saturation of the bean tissues for virus was but 2/3 or less that of the control, other factors being equal. Thus the first points for the normal serum and antiserum curves are proportionally higher than they should be

on the basis of pure serum effect alone. No correction has been attempted for this error, which is not usually observed beyond the first or second points of the dilution scale, but an appreciation of its nature and significance furthers an interpretation of the essential parallelism of the normal serum and control curves, a parallelism that is interpreted as indicating that normal serum decreases the infectivity of the virus involved through a lowering of the susceptibility of the host to the virus, not through any detectable effect on the virus itself.

Passing now to the immune serum curve, we observe that it starts from a point essentially coincident with that of the normal serum curve at  $1/2$  virus dilution; and then, as the absolute amount of virus decreases, the immune serum curve rapidly falls off, fast approaching zero at a virus dilution of 1:200, a result attributed to a direct or indirect effect of immune serum on the virus itself. If the host-tissue saturation error as applied to the normal serum curve also be applied in the same way to the immune serum curve, the first portion of the latter will be much more nearly horizontal, and the curve will tend to approximate even more closely the theoretical curve for a substance affecting virus. As one might expect, antiserum has in addition the same host effect as normal serum, hence it approaches the horizontal position of the normal serum curve as a limit, not that of the control virus curve.

It will be noted that the aspecific host effect of normal serum is essentially of the same type as that of ovalbumin (figure 1). This same phenomenon has been tested for a number of inhibitory substances unrelated to the virus, *e.g.*, milk, normal tobacco extract, and found in each case to be the same type as the effect due to normal serum. The egg-albumin curve resulted from the use of this protein at a dilution of 1:200. Tobacco extract, on the other hand, must be used at relatively high concentration (1:5 or less) to display a corresponding effect. The fact that tobacco extract has thus been shown to have an effect in lowering the susceptibility of the bean to tobacco-mosaic virus demonstrates the validity of regarding the presence of tobacco products in virus saps and extracts as one of the factors (see above) involved in the flattening of the normal curve for tobacco-mosaic dilutions tested for infectivity on bean leaves.

Turning next to the question of the inherent variability of the reactions described, reference to figure 2 shows at a glance a repetition of the same type of experimentation applied to a number of types, strains, and separate preparations of virus, using different lots and species of test plants, different normal and immune sera at different dilutions, different diluents, and performing the reactions at different times. Consistently throughout one sees that, although there is variation in slope and point of origin of the descending curve for immune serum and in the smoothness of the parallel-

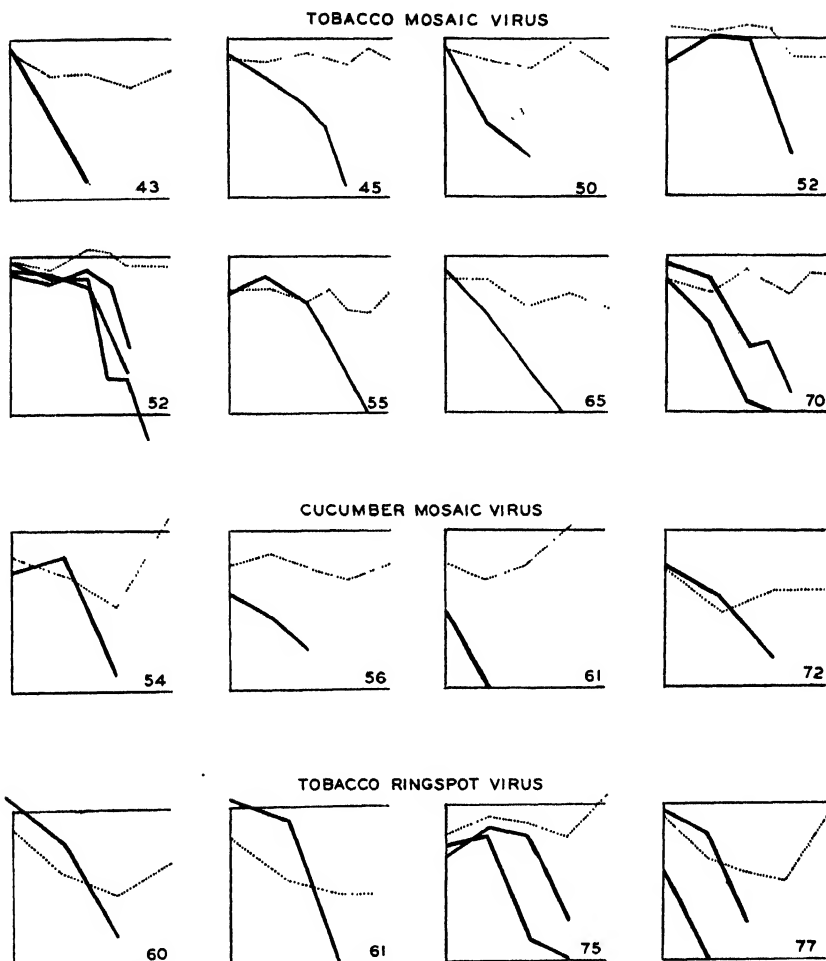


FIG. 2. Examples of normal serum and immune serum inhibitions of tobacco-mosaic, cucumber-mosaic, and tobacco-ring-spot viruses. In each case the upper solid line represents the virus control, the dotted line the normal serum reaction, the heavy line the immune serum reaction. Abscissa, virus dilution; ordinate, percentage of infectivity in terms of virus control. The numbers refer to the protocols involved. Further discussion in the text.

ism of the normal serum curve, there is a constant tendency for normal serum to lower the control curve to a greater or less extent, while roughly preserving its horizontal character, and for the immune serum curve to maintain the parallelism with the control for only a short distance before commencing an abrupt fall.

Since it has been noted that the immune serum curve has a tendency to parallel the control curve for some distance before its rapid fall (see

Experiments 52, 55, and 75 in Fig. 2), the question might arise as to whether the normal serum curve is essentially different from the antiserum curve, whether the normal serum curve is merely parallel to the control curve

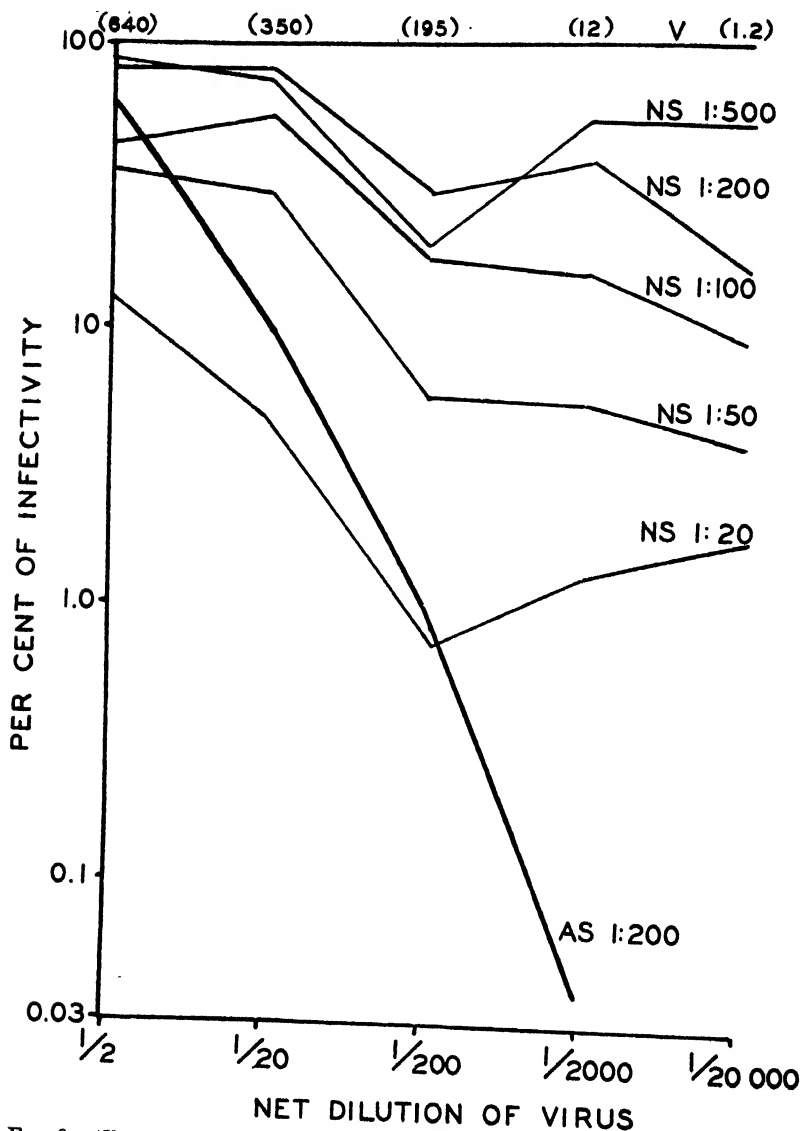


FIG. 3. The effect on virus infectivity of varying the concentration of normal serum. Normal serum (NS) at dilutions as indicated, immune serum (AS) for comparison, all in terms of control virus (V), the absolute values of which are given above. Percentage of inhibition as determined by infectivity counts on bean. Discussion in the text.

because the point of its rapid fall has not been reached in the dilutions of virus thus far used; in a word, whether the reactions of immune serum and normal serum are not inherently the same, both being effects on the *virus*, but differing in degree. The possibility appears reasonable, because, as will be shown below, a decrease in the amount of antiserum used lengthens the horizontal portion of the antiserum curve before its abrupt descent, although the ultimate descent appears inevitable. An experiment was performed in order to demonstrate whether or not the normal serum curve retains its parallelism to the limits of experimental possibility.

In the experiment in question the effect of normal serum was tested in concentrations far exceeding those used in routine practice. The results are shown in figure 3. Here one sees that even if normal serum is used in concentration ten times as strong as in previous experiments, the curve resulting is still essentially parallel to the control curve. It may be noted that as the concentration of normal serum increases and the absolute number of lesions produced consequently decreases, the steepness of the slope

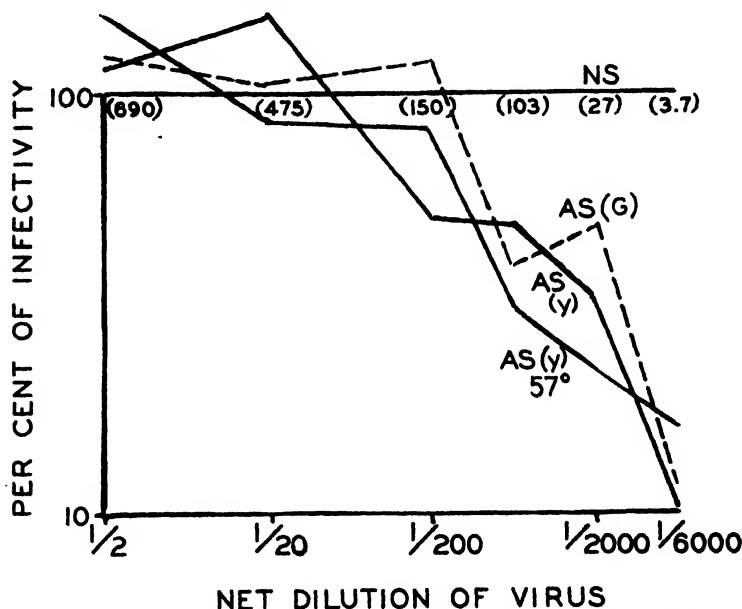


FIG. 4. Effect of complement and of variation in strains of virus employed on neutralization of tobacco-mosaic virus by immune serum. Infectivity of immune serum (AS) expressed in terms of virus plus normal serum (NS) at same dilution, as determined from infectivity counts on bean. Discussion in the text. The virus employed was a white strain of tobacco mosaic, the two antisera were respectively: AS·Y, an antiserum to the same white strain as was used in the experiment; AS·G, a heterologous antiserum immune from the ordinary green strain of tobacco mosaic. Illustrative of the lack of participation of complement in neutralization, and of nonspecificity as between different strains of the same virus type.

due to saturation of leaf tissues with virus increases, which is in conformity with the above interpretation of this portion of the curve.

A further point to consider regards the rôle of complement in the reaction that we may now consider the neutralization of virus by immune serum. Miss Purdy (9), using a technique less sensitive than the one used in the present work, reported that immune serum inactivated for  $\frac{1}{2}$  hour at  $57^{\circ}$  C. was less active in inhibiting the infectivity of tobacco-mosaic virus than unheated serum, and that this loss in inhibitory power was restored on the addition of serum-complement. In order to test this point an experiment was performed in which a portion of antiserum was heated to  $57^{\circ}$  C. for  $\frac{1}{2}$  hour before testing for inhibitory effect. As will be seen from the accompanying graph, figure 4, one observes no essential difference in inhibitory power of the heated as compared with the unheated serum.

This lack of participation of complement in the neutralization reaction of immune serum is consistent with the findings as regards the animal

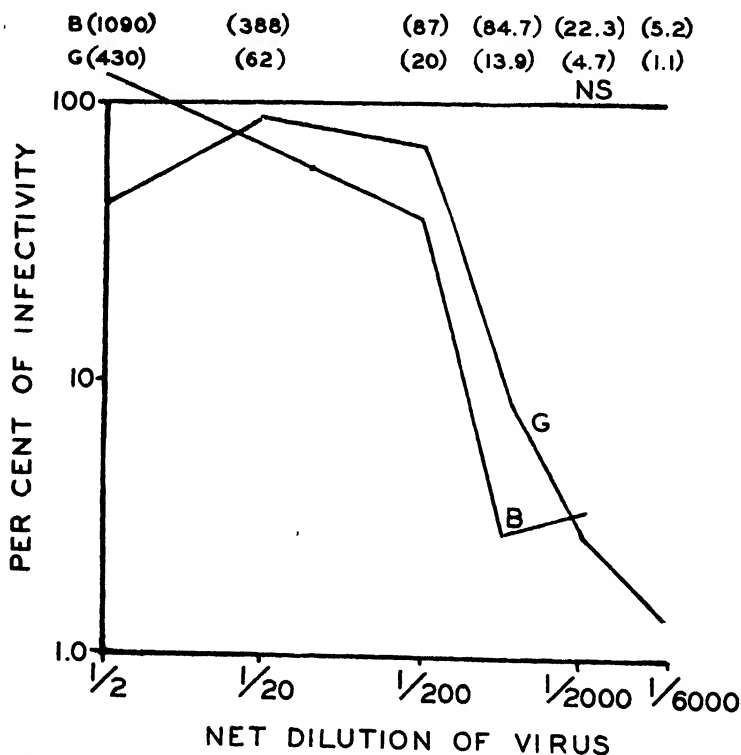


FIG. 5. Percentage of inhibition of tobacco-mosaic virus by anti-tobacco mosaic serum at 1:200 in terms of normal serum control (NS) as measured by infectivity counts of the same preparations on beans (B) and on *Nicotiana glutinosa* (G). Discussion in the text.



viruses. There is strong evidence in the literature that complement is in general unnecessary for the demonstration of neutralization, as for example in connection with the viruses of rabies and vaccinia.

If, as has been indicated, the inhibitory effect of normal serum is on the host plant, while immune serum in addition has an effect on the virus, further information might be obtained by studying the behavior of the same serum-virus mixtures when tested on different types of host plants. Figure 5 gives the results of such a test, the host plants used being the bean and *Nicotiana glutinosa*, two plants widely separated in systematic position. It will be seen from the curves obtained that the action of immune serum in inactivating virus is essentially the same on both of these plants, which is indicative that we are dealing with a reaction that occurs *in vitro*.

Briefly stated, the present section indicates that the inhibitory effects of normal serum and immune serum are qualitatively different, that normal serum shares with egg albumin, milk, and tobacco extract the quality of affecting the host plant in such a way as to lower its susceptibility to virus to an equal fractional extent regardless of the amount of virus applied. Immune serum, on the other hand, beside sharing this host-affecting property of normal serum, also has an effect on the virus, inactivating it partially or completely, according to the proportions of virus and serum present.

#### Quantitative Relationship of Serum to Virus

If antiserum reacts directly on virus, inactivating the latter, then it should be possible to demonstrate a quantitative relationship governing the reaction. Such has been done with respect to tobacco-mosaic virus, and a typical experiment is shown in figure 6. In this case a lead acetate purified supply of ordinary tobacco-mosaic virus was employed in dilution series and to each dilution was added a constant amount of antiserum dilution, the dilutions employed being 1/20, 1/200, and 1/2000. Normal serum at 1/200 was used for control of the customary host effect, and the percentages of infectivity of the mixtures as compared with the virus control plotted as in the figure. The horizontal portion of each curve represents the portion in which there is such an excess of virus that neutralization has not yet become evident, and hence the difference between this horizontal portion and the virus control gives a measure of the pure host effect of all sera. This effect is greater as the concentration of the serum increases, as previously noted in figure 3. The parallelism of the three curves is strong evidence for the occurrence of the same degree of reaction when neutralization finally appears, and the point of this appearance gives an index of the point at which the virus quantity has been sufficiently reduced to show neutralization in the presence of the amount of serum added (supplementary experimentation has shown that the curve for antiserum at 1:20 be-

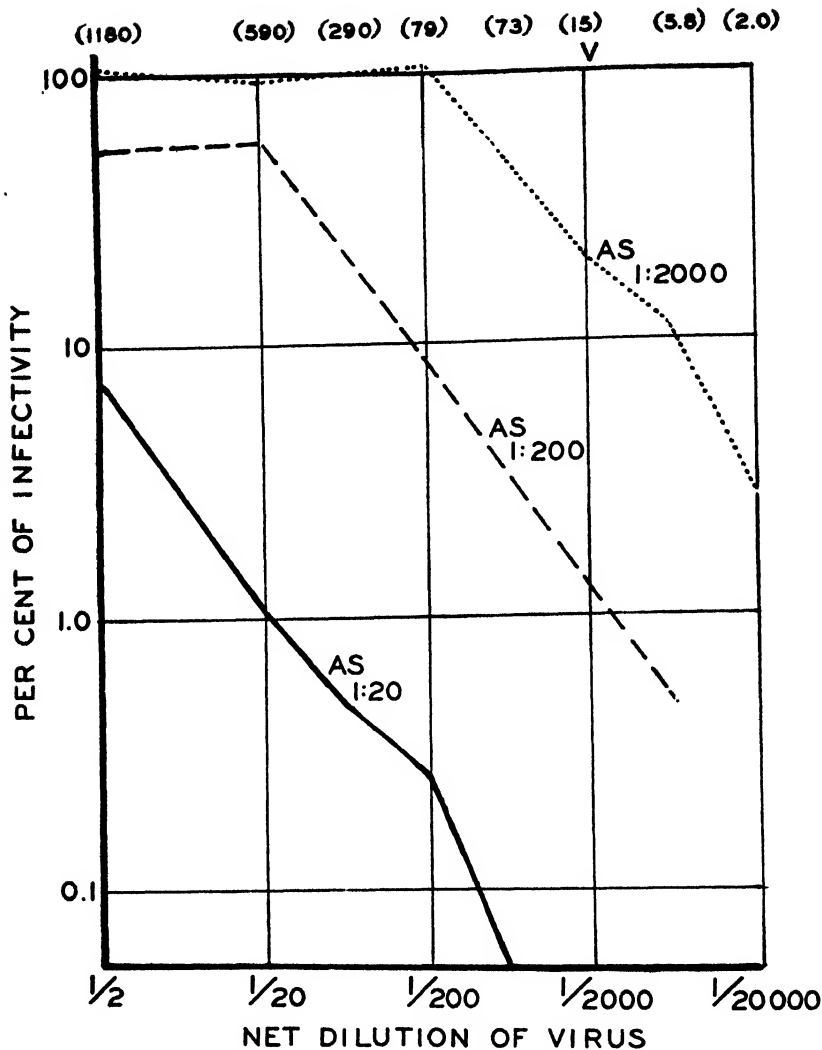


FIG. 6. Quantitative relationship of anti-tobacco mosaic virus serum to tobacco-mosaic virus. Antiserum (AS) at dilutions as indicated compared in infectivity with serum-free control (V). Arrangement as in preceding graphs. Discussion in the text.

comes horizontal at or near the virus dilution 1:2). A comparison of the points of departure of the curves from the horizontal shows that if the concentration and hence the absolute amount of the serum be increased tenfold, then the reaction first appears when the amount of virus present is tenfold, and if the amount of serum be reduced by a factor of 10, then it is able to neutralize only 1/10 as much virus, i.e., the amount of virus neutralized varies directly as the amount of immune serum added. If the amount

of virus be doubled, twice as much serum must be added in order to produce the same neutralizing effect. This is a phenomenon entirely independent of any effect on the host plant, since in each curve the relationships of serum to host plant have been maintained constant, only the quantity of virus being varied. This experiment has been repeated using different serum dilutions, and with essentially the same results. We thus see that the interaction of immune serum with tobacco-mosaic virus is governed by a law of simple proportionality, which fact may be best interpreted as indicative that we are dealing with a true reaction on the virus.

#### The Time Factor in the Inhibition of Tobacco-Mosaic Virus by Normal and Immune Sera

With neutralization of the animal viruses by specific sera, a certain time of incubation of the serum-virus mixture is customarily considered necessary for adequate demonstration of the neutralizing action. With the plant material at hand it was accordingly of interest to determine the part played by incubation time, if any, on the two reactions before us. Preliminary experiments having indicated that in even so short a time as 5 minutes the neutralizing effect of immune serum is readily demonstrated, it seemed desirable to center attention on even shorter intervals. Accordingly the following experiment was performed.

A highly potent purified preparation of tobacco-mosaic virus was subjected to the action of the most potent immune serum available, and at the same time to that of the normal serum originating in the same animal. One set of virus dilutions was mixed with equal amounts of normal and immune serum, respectively, incubated at room temperature for 1 hour, then plated out on bean leaves, which latter were thoroughly washed exactly 1 minute after inoculation. A second set of the same virus dilutions was mixed with equal amounts of the same sera, incubated at room temperature for 4 minutes, then plated out on beans, the leaves of which were also washed after 1 minute. A third set of the same serum-virus mixtures were shaken thoroughly after mixing and inoculated at once, the leaves being washed after 1 minute as before. In the case of these last, momentary mixtures, in order to avoid too much delay between mixing and inoculation, several separate identical mixtures were successively used whenever the number of pots of beans to be inoculated exceeded 3. The time between inoculation and washing was controlled by a stop-watch. The inoculation of 3 pots of beans required about 2 minutes, hence this represents the maximum time consumed in the case of the "nonincubated" mixtures. The three lots thus were incubated respectively: 1 hour  $\pm$  1 minute; 5 minutes  $\pm$  1 minute; and 1 minute  $\pm$  1 minute; and were all washed exactly 1 minute after the inoculation.

The results of this experiment with regard to the immune sera are given in figure 7. It is seen that if the incubation time is shortened from 1 hour

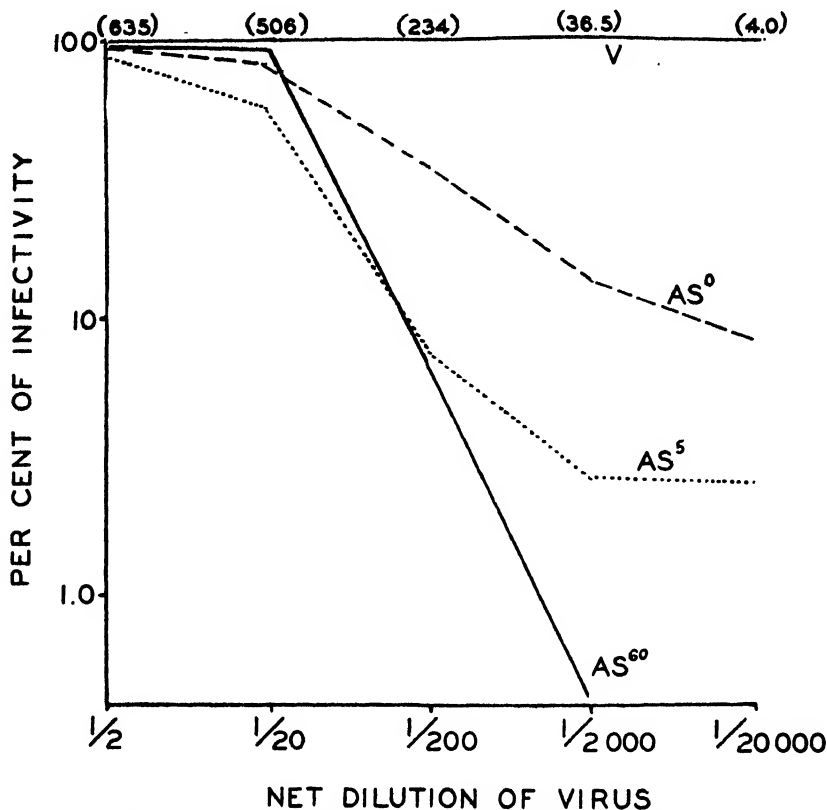


FIG. 7. Effect of time on the neutralization of tobacco-mosaic virus (V) by immune serum (AS). AS<sup>0</sup>: Serum-virus mixture plated without incubation; AS<sup>5</sup>: Serum-virus mixture incubated 5 minutes at room temperature, then plated out; AS<sup>60</sup>: Serum-virus mixture incubated 1 hour before plating out. Illustrative of the dependence of neutralization on time *in vitro*. Discussion in the text.

to 5 minutes there is a considerable loss in effectiveness of neutralization, and if this time is further shortened as much as possible, there is additional loss in reaction, although, because of the time necessarily involved in the process of inoculation, it was impossible to show whether the reaction can be completely eliminated. The corresponding mixtures of normal serum and virus showed no essential difference in reaction regardless of whether the time of incubation was 1 hour or momentary.

This experiment shows that the reaction of immune serum with virus is a reaction that occurs *in vitro*, since it requires time *in vitro* for its full accomplishment. Such a finding is entirely consistent with the view that

the inhibitory effect of immune serum is a reaction affecting the virus, independent of any detrimental effect on the host plant. The fact that the time between 2 minutes or less and 1 hour has no appreciable effect on the normal serum reaction likewise indicates that the reaction of normal serum is a reaction that does not take place *in vitro*, a finding that is again consistent with the view that the reaction of normal serum is a reaction on the host plant, nor on the virus.

So far as has been observed, the reaction between immune serum and virus is largely accomplished during the first 10–15 minutes of incubation at room temperature. The degree of reaction after 1 hour's incubation at room temperature is not appreciably increased by increasing the incubation time to as much as 36 hours, including 2 hours at 37° C. On the other hand, as figure 7 shows, the reaction after 2–5 minutes is by no means complete.

#### Specificity of the Neutralization Reaction

In order to study the neutralization of a virus by immune serum it is necessary to have some fairly accurate method for measuring the infectivity of virus samples. Tobacco mosaic is ideal for this purpose, because concentrated virus preparations produce as many as 1600 or more local lesions on the bean leaf and because such virus may be diluted as much as 1:10,000,000 without total loss of demonstrable infectivity. Thus it was inevitable that the greatest part of the work reported in the present paper with regard to the nature and properties of the reactions studied was carried out using tobacco-mosaic virus. Only two other of the viruses available have as yet yielded even partially to attempts at accurate quantitative methods, these two being tobacco ring spot and cucumber mosaic. Tobacco ring spot, if plated out on the cowpea, may give as many as 60 or more lesions per leaf, using undiluted expressed sap as inoculum, and is infectious to cowpea in dilutions up to 1:125, cucumber mosaic is much less infective, an average of 10 lesions per leaf with concentrated virus being exceptional, and dilutions infective to 1:100 rather rare. Often, because of physiological factors not yet understood, whole experiments must be rejected because of an infectivity too low to yield significant results. For these reasons the experiments described below have not yielded evidence as extensive and invariable as that from the study of tobacco mosaic; nevertheless, the experiments have been repeated a sufficient number of times to insure that the general trend of the results is significant.

That the neutralization reaction with the plant viruses be specific is not a necessary concomitant of its being a true serological reaction. With bacteriophage, for example, there is considerable disagreement as to the specificity of the neutralization test. Yet, with many of the animal viruses, the neutralization reaction is delicately specific, this specificity even extend-

ing to strain distinctions as in the case of the various strains of the foot-and-mouth-disease virus. It has, therefore, been of particular interest to test the specificity of the neutralization reaction with the three plant viruses available and with their various strains.

If a series of dilutions of tobacco-mosaic virus is treated with constant amounts of immune serum for tobacco mosaic there results an inactivation characterized by a sharply falling neutralization curve, as has been pointed out above. If at the same time the same virus dilutions are treated in the same way with the same concentrations of comparable antisera against cucumber mosaic or tobacco ring spot, these latter immune sera behave in no way different from normal serum with respect to the tobacco-mosaic virus. This situation is illustrated by the results of a typical experiment

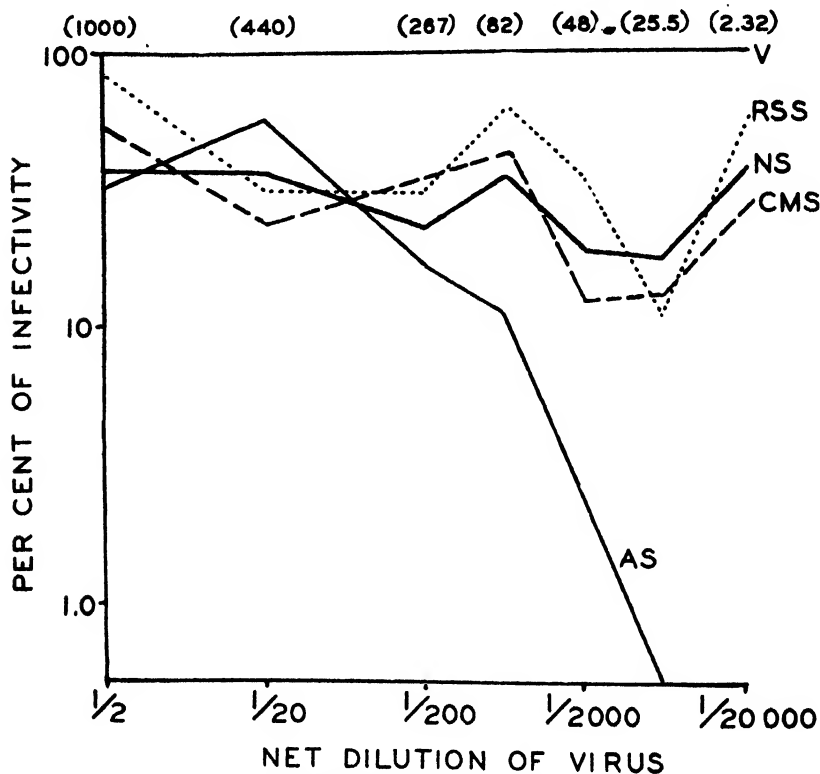


FIG. 8. Specificity of neutralization with tobacco-mosaic virus subjected to treatment with normal serum (NS), tobacco-ring-spot serum (RSS), cucumber mosaic serum (CMS), and anti-tobacco-mosaic serum (AS), all at dilutions of 1:200 and all expressed in terms of serum-free control (V). Percentage of infectivity determined by lesion counts on bean. The same sera were used in figures 8-10, and these sera were all produced in similar manner from the injection of rabbits with expressed Turkish tobacco sap containing the viruses in question.

shown in figure 8, an experiment that has been several times repeated using different sera and virus preparations and always with essentially the same results.

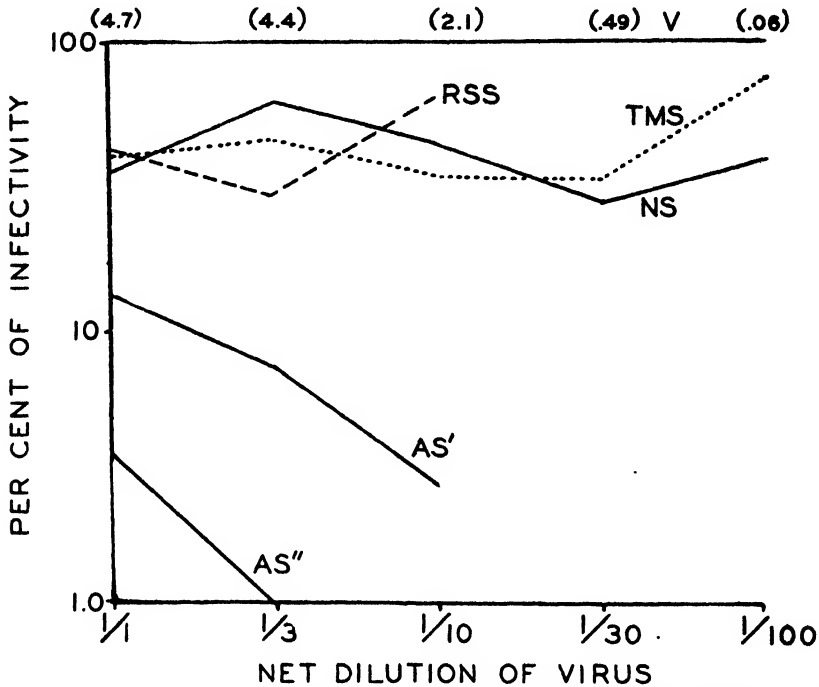


FIG. 9. Specificity of neutralization of cucumber-mosaic virus subjected to treatment with tobacco-mosaic serum (TMS), tobacco-ring-spot serum (RSS), and with two anti-cucumber-mosaic sera (AS', AS''), all at dilutions of 1:50 and all expressed in terms of infectivity of untreated virus control (V) at the same dilutions. Percentage of inhibition determined by infectivity counts on cowpea. Further discussion in the text.

If we now treat cucumber-mosaic virus with the same three sera, only the anti-cucumber-mosaic serum causes a falling curve, the other two sera behaving just as normal sera (Fig. 9). If finally we use tobacco-ring-spot virus, this virus becomes inactivated only by the anti-tobacco-ring-spot serum, not by the anti-cucumber-mosaic or anti-tobacco-mosaic sera (Fig. 10).

Thus, of the three virus types before us, tobacco-mosaic virus is inactivated only by anti-tobacco mosaic serum, cucumber-mosaic virus only by anti-cucumber mosaic serum, and tobacco-ring-spot virus only by anti-tobacco-ring-spot serum—the cross specificity is absolute, and the addition to any of these viruses of a heterologous antiserum exhibits no effect different from the host effect associated with all sera, normal or immune.

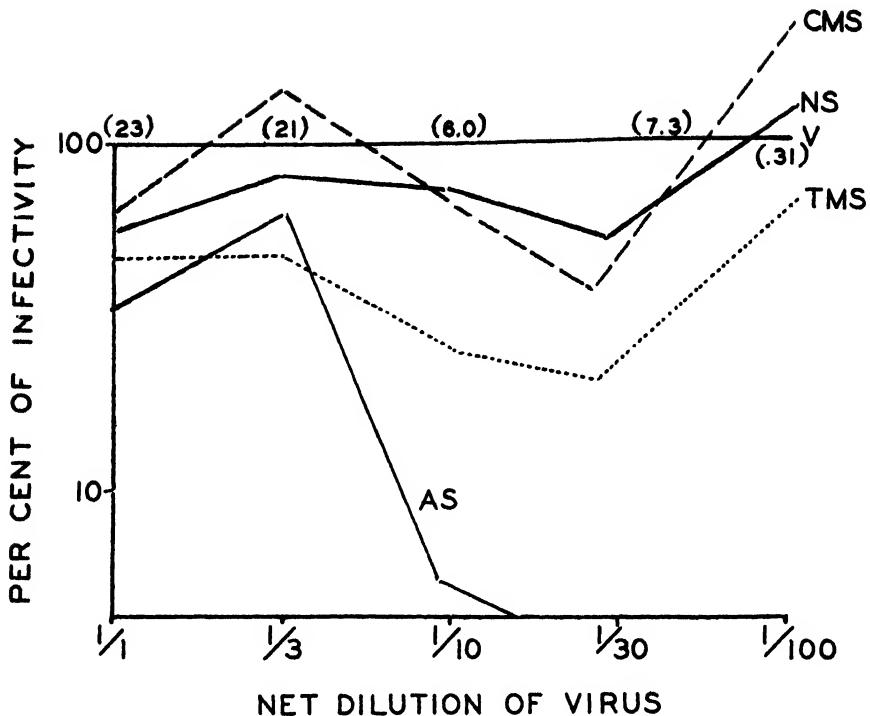


FIG. 10. Specificity of neutralization of tobacco-ring-spot virus subjected to treatment with tobacco-mosaic serum (TMS), cucumber-mosaic serum (CMS), anti-tobacco-ring-spot serum (AS), and normal serum (NS), all at dilutions of 1: 50 and all plotted in terms of the infectivity of the serum-free control (V). Inhibition measured by infectivity counts on cowpea. Discussion in the text.

Among the strains of any one of the three virus types studied, the evidence available up to the present indicates that such strains as have been tested are serologically very closely related. This subject will be mentioned only very briefly since the experiments involved are still being continued. Suffice it to say that with cucumber mosaic and tobacco mosaic no serological differences in strains of the same virus type have as yet been noted. With the apparently very different yellow and green strains of cucumber mosaic used, complete cross tests have not been performed as yet, but all of the data on cucumber mosaic given above have been obtained from tests of antigreen cucumber-mosaic serum with solutions of the yellow strain of virus. That a specific reaction does occur here evidences the close serological relationship of these two strains in spite of the very different symptoms produced.

With tobacco mosaic, cross neutralization tests using brilliant white, ordinary, and symptomless strains, have yielded no evidence as yet that



these remarkably different appearing strains possess any serological distinctness from one another. Figure 4 illustrates a typical experiment of this sort. The virus used was the white strain of tobacco mosaic "Jensen 108." It is seen that when treated with antiordinary or antiwhite tobacco mosaic serum the results are essentially the same in both cases, either antiserum neutralizing the white virus to approximately the same degree. The reciprocal test gave essentially the same results.

It is remarkable that certain yellow strains of tobacco mosaic and cucumber mosaic are at times indistinguishable on tobacco and capable of being confused by the connoisseur. Differences in host range and other properties have classified these virus strains in their present positions. The serological evidence entirely confirms that obtained from a study of host range and other properties in showing that the yellow strain of cucumber mosaic employed is closely related to the very different appearing green strains of cucumber mosaic, while the same yellow strain of cucumber mosaic is wholly unrelated to a superficially indistinguishable yellow strain of tobacco mosaic.

#### SUMMARY

It is known from the work of earlier investigators that normal rabbit serum decreases the infectivity of tobacco-mosaic virus and that anti-tobacco-mosaic serum shows a greater inhibitory effect. This phenomenon has been further studied with the following results:

A technique is described which permits the differentiation of virus-inhibitory effects acting through decreasing the susceptibility of the host plant, and inhibitory effects due to inactivation of the viruses.

Using this technique it is shown that normal rabbit serum inhibits virus infectivity through decreasing the susceptibility of the host, not through an effect on the virus. This property is shared by other nonspecific proteinaceous substances, *e.g.*, healthy tobacco extract, ovalbumin, and milk.

Virus-immune serum, beside showing the same, non-specific host effect as normal serum, also exerts a specific neutralizing action on the virus used in immunization.

Neutralization of the virus of tobacco mosaic follows a law of multiple proportions: if the amount of virus be increased by a given percentage, the amount of immune serum added must be increased by the same percentage in order to produce the same inhibitory effect.

The host-inhibitory effect of normal serum is independent of time *in vitro* while neutralization of virus by immune serum requires time *in vitro* for its accomplishment.

There is an absolute cross specificity in the neutralization of the viruses

of tobacco mosaic, tobacco ring spot, and cucumber mosaic, such that each of these viruses is completely neutralized by its homologous serum, but not at all by the heterologous sera. As far as has been tested at present this specificity does not extend to virus strain distinctions.

The experiments show that the reaction in question is a specific, quantitative neutralization of virus by immune serum, such as has not heretofore been described for the plant viruses. The reaction has been used in the determination of the relationships of certain plant viruses and offers possibilities of further application in the study of the fundamental problems of plant virus research.

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# INVESTIGATIONS ON PHYSIOLOGIC SPECIALIZATION OF *TILLETIA LAEVIS* IN KANSAS<sup>1</sup>

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## INTRODUCTION

The bunt problem in the Great Plains has become very important during the last 8 years. Early records of wheat production in Kansas indicate that bunt, caused by *Tilletia laevis* Kühn, was comparatively uncommon and that from 1900 to 1917 very little seed wheat was treated for its control. During that period strains of Turkey wheat (Crimean) were commonly grown in Kansas. Since then new varieties of hard red winter wheats (Blackhull and Kanred) have been extensively grown and, while it is true that they are similar to the original Turkey, they are much more bunt-susceptible. Coincident with the growing of these new varieties, the bunt problem became one of tremendous importance in Kansas, where, in 1926, the loss caused by this disease was estimated at about \$14,811,000 (13).

Whether the epiphytotics of bunt that have occurred in the Great Plains since 1925 are due more to changes in the varieties of wheat, or to variations in the fungus, *Tilletia laevis*, is speculative. It seems probable that both of these factors, as well as seasonal conditions and the wider distribution of smutty seed wheat, are involved.

## PHYSIOLOGIC FORMS OF BUNT AND THEIR SIGNIFICANCE

It has been known for several years that physiologic forms occur in bunt and other smuts (4, 12, 15, 17, 20). Recent investigations (1, 2, 6, 7, 8, 9) have shown that there are several physiologic forms of both *Tilletia tritici* (Bjerk.) Wint. and *T. laevis*. Reed (15) and Roemer (19) have proved that collections of bunt from various States in the United States and from Hungary, Egypt, Italy, Norway, New Zealand, Czechoslovakia, Austria, England, and Germany are composed of various physiologic forms of *T. tritici* and *T. laevis*. It also has been clearly demonstrated that there is a vast difference in the reaction of varieties of wheat to bunt collected from different localities. Certain varieties that at one time were regarded as resistant to bunt are no longer resistant (2). Flor (6) states that

<sup>1</sup> Contribution No. 337 from the Department of Botany, Kansas Agricultural Experiment Station.

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certain varieties maintained their resistance for a number of years, but are now being attacked with increased severity from year to year.

Studies on heterothallism and hybridization in the bunt fungi (6) show that the possibilities of the origination of new and more virulent forms of *Tilletia tritici* and *T. laevis* are unlimited. In the light of present knowledge mutations in the bunt organisms may also be expected. Certainly, the bunt organism must be regarded as more or less unstable in its pathogenicity when one considers that sporidial fusion (5) is necessary to the production of a smutted spikelet of wheat. Hybridization, therefore, frequently occurs and many of the chlamydospore collections used in infection studies in the field are essentially hybrids. Several investigators now consider chlamydospore collections as physiologic forms, although there are arguments to be raised against this opinion. This point of view, however, is followed in this paper.

Because of variations in the fungus, the search for resistance to bunt in winter and spring wheats has become not only greatly complicated, but it is now realized that in most cases a resistant variety, if secured, will be only a temporary attainment. In some of the tests for varietal resistance to bunt by artificial inoculation, only one or two collections of bunt have been used. These would not be representative of the bunt flora over a wide area. This criticism has been suggested by other workers (2, 4). In testing wheat varieties, selections, and hybrids for their reaction to bunt, the collections of smut used for inoculation should be secured from several counties in a State, or even from wheat-growing areas in several States. Whether state or even national boundaries should limit the source of inoculum will depend upon the area to which it is expected or hoped the new variety may be adapted. The reaction of a variety at one station to one form of bunt may be a very poor criterion of its behavior over a wider area.

The complexity of the situation may be realized when one considers that, theoretically, a single smutted head of wheat may contain not only both species of *Tilletia* but, also, several physiologic forms each of varying degrees of pathogenicity; that each chlamydospore represents a diploid phase of the fungus; and that sporidial fusion has taken place before a smut ball was produced.

There is, however, abundant evidence that varieties of commercial value can be produced that show marked resistance to bunt in the field, such as Ridit (3, p. 932), Albit, Oro, and the Turkey selections recently made at the Nebraska station (11). The ability of these varieties to remain resistant may be limited to a comparatively short time, however; in fact, some of them are now known to be susceptible to some forms of bunt. If a commercial variety, however, remains resistant for a period of 5 to 10

years, there is ample justification for all the work required to obtain such a variety, and investigators should realize the necessity of continuous work on this problem. There are numerous cases among the smut fungi (18) in which profound changes have occurred in the host-organism relationship. One needs only to consider what has taken place with Ridit, Oro, and Martin (2), and some of the "resistant" durum wheats and Marquis with respect to bunt (10), Kanota oats and other resistant *Fulghum* selections with respect to oat smut (15), and milo, *feterita*, and some of the other resistant sorghums in regard to kernel smut (14).

#### PURPOSE OF EXPERIMENTS

The primary objective of these experiments was to prepare the way for the production of a desirable bunt-resistant hard winter wheat. In order to gain some knowledge of the bunt flora of Kansas, a number of collections of bunt have been made from several counties. These have been studied on a group of wheat varieties in order to separate the smut collections into what may be called physiologic forms. An attempt has been made to learn the distribution of these forms in Kansas. A study has been made also of certain of these forms on artificial media. Some inoculation experiments have shown that resistant varieties may act as media for building up more virulent physiologic forms of bunt. This process has been described by other investigators (21).

The studies here reported are not considered final; in fact, it is probable that other physiologic forms could be demonstrated if larger numbers of collections of bunt from all counties and regions of Kansas were made and if more varieties of wheat were used as differential hosts. It also is recognized that assigning certain bunt collections to a definite form number is not always possible. Furthermore, the form number assigned may in some cases be incorrect. The point that the writer wishes to emphasize is that distinct bunt strains of differing virulence exist in Kansas. A knowledge of their prevalence and distribution is of importance to those engaged in wheat improvement. Plant pathologists and plant breeders should not fail to give careful thought to this matter in making and carrying out plans for producing new varieties of commercial value. The grave mistake of distributing a new variety without knowing its reaction to the important diseases that may attack it has been made too frequently in the past. Aamodt (1) mentions the danger attending the introduction and growing of spring varieties of wheat such as Kota, Ceres, and Progress, even though they are resistant to stem rust. The bunt reaction of these varieties had not been determined prior to their introduction. In this connection he says, "these new varieties of wheat then, together with the durum wheats, have greatly aggravated the bunt problem, not only because of the inability

of the varieties to withstand attacks by the bunt organism, but because they have been a medium through which the pathogene has become more thoroughly and widely distributed."

#### METHODS AND MATERIALS

*Bunt collections were made in Kansas in 1928 to 1931, inclusive, on susceptible commercial varieties of wheat, such as Kanred, Blackhull, and Turkey. All these collections of bunt proved to be *Tilletia laevis*. The standard varieties of wheat used in these physiologic-form studies are winter wheats, planted in October of one year and harvested the following June.*

The collections of smut were carefully prepared and each was handled as though it might be a distinct physiologic form or aggregate of forms. Generally, 2 sowings of the complete list of varieties were made from inoculated seed on 2 dates. One planting was made late enough in the fall to coincide with optimum conditions for infection. The seed of the standard varieties was disinfected before inoculating with the bunt spores. The ordinary methods for applying maximum spore loads were used, care being taken to insure that each variety received approximately the same amount of inoculum. The bunt nursery consisted of 5-foot rows planted a foot apart. The plantings in the bunt nursery for a given seeding date were all made simultaneously. During the 4 years in which these studies were made, the soil temperature range for a week following planting was 35° to 60° F. This range proved satisfactory and heavy infections resulted. When harvested each row was cut separately, tied, and labeled. Each head was examined for bunt. Percentage of bunt was determined on the basis of counts of smutted and normal heads. The total number of wheat heads per row ranged from 200 to 475.

The standard varieties used in these studies were selected from a list of many that had been reported to have differential reactions. Of the 12 varieties used, only 6 were finally selected as physiologic-form differentials. The standard varieties used were Banner Berkeley (C.I. 7362), Hussar (C.I. 4843), Oro (C.I. 8220), Ridit (C.I. 6703), Turkey (C.I. 1558A), Martin (C.I. 4463), White Odessa (C.I. 4655), Kooperatorka (C.I. 8861), Turkey × Bearded (Minnesota No. 48) (C.I. 8243), Yogo (Minturki × Beloglina × Buffum) (C.I. 8033), Regal (C.I. 7364), and Kanred (C.I. 5146). Of these the first 6 were used as differentials.

#### SOIL INFESTATION OF BUNT

One of the reasons why bunt has not become a more serious limiting factor in wheat production in Kansas is that soil infestation by bunt spores does not occur. There has been no positive evidence in the past 20 years

## BUNT-COLLECTING CENTERS IN KANSAS

[illegible]

FIG. 1. Maps of Kansas. Upper map shows sources of bunt collections: A, 1928; B, 1929; C, 1930; D, 1931. Lower map shows distribution of physiologic forms of bunt.

tions were made in the principal winter-wheat counties of central and western Kansas. The varieties listed in the tables are arranged from left to right in the same order they were sown in the nursery. The reaction of each variety to the various collections of bunt, expressed in percentage of bunted heads, is given in the column under each variety.

In table 1 the county where the collection of bunt was made, the collection number, and the year are given. Since Kanred proved to be very susceptible to all bunt collections, the bunt present on this variety was saved each year as inoculum for all varieties in the next year's test. The results are listed separately for the various varieties tested. A total of 64 collections of bunt from widely scattered areas in the State were used in the course of these studies. In general, the reactions from year to year for the same collection were very similar. It is evident, however, that some collections of bunt did not infect the differential varieties to the same extent each year. This is to be expected, since a field collection may contain several forms all of which may vary in amount in the succeeding years.

There are distinct differences in pathogenicity to be noted in several of the bunt collections. Banner Berkeley has been recognized as a resistant variety, and, for the most part, it is resistant to the bunt collections made in Kansas. This is not true, however, for such collections as Brown County No. 17, Greeley County No. 19, Johnson County No. 8, and Lane County No. 53, since Banner Berkeley had 59.5, 19.4, 59.8, and 20.3 per cent of bunt, respectively, from these collections. Hussar, which also is usually highly resistant, produced 14.1 per cent infection in the case of Meade County No. 23 collection. Martin was highly resistant to most of the bunt collections used in these studies, but several collections were decidedly virulent on this variety, as, for example, Brown County No. 17, Johnson County No. 8, and Meade County No. 23, which gave 39.7, 39.6, and 14.4 per cent of bunt, respectively. Oro was highly resistant to all collections except Lincoln County No. 56, which produced 8.7 per cent infection. Redit was markedly resistant to all collections except Barton County No. 2, Clark County No. 3, and Meade County No. 23, where moderate infections of 11.2, 10.4, and 8.9 per cent, respectively, were produced. Turkey Sel. No. 1558A was one of the most consistently resistant varieties subjected to the Kansas bunt collections. As will be shown later, however, such infections on the Turkey selection as 8.9 per cent smut in Edwards County No. 49 and 14.4 per cent in Pratt County No. 14 are significant differences.

There also are marked differences in the reaction of White Odessa, Kooperatoroka, Turkey  $\times$  Bearded, Yogo, and Regal to the various bunt collections. These varieties were not used in constructing the key for the separation of the physiologic forms; however, some of these wheats could have been so used. In contrast to the resistant varieties discussed, it is of



TABLE 1.—*Reaction of a selected group of wheat varieties to collections of bunt, Tilletia laevis, made in various counties in Kansas, and tested at Manhattan during the period, 1929 to 1932, inclusive*

Source of collection	Collection No.	Year		Percentage of heads bunted											
				Banner Berkeley	Hussar	Oro	Ridit	Turkey 1558A	Martin	White Odessa	Kooperatorka	Turkey x Bearded	Yogo	Regal	Kanred
		Col-lected	Grown												
County															
Barton	1	1928	1929	0.0	0.0	3.1	5.7	0.2	0.0	0.2	0.4	0.0	2.8	0.4	61.9
"	2	"	"	0.0	0.0	0.6	11.2	0.0	0.0	0.0	1.5	0.0	2.2	2.5	59.4
"	16	1929	1930	0.0	0.0	0.0	0.4	0.0	0.0	0.0	1.0	2.5	0.4	0.0	44.5
"	"	"	1931	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.8	0.0	47.1
"	"	"	1932	0.7	0.0	1.4	0.0	2.2	0.0	0.0	0.0	0.0	2.0	0.0	22.1
"	37	1931	"	0.0	0.0	0.7	0.4	1.2	0.0	0.0	0.8	0.0	2.1	0.0	27.5
"	38	"	"	0.0	0.0	0.4	1.1	2.4	0.0	0.0	0.7	0.9	0.6	0.2	12.7
"	39	"	"	1.2	0.0	0.0	0.4	0.4	0.0	0.0	0.4	0.0	2.8	0.0	61.7
Brown	17	1929	1930	23.1	0.0	0.0	0.0	1.0	2.2	2.1	4.3	1.0	0.0	2.5	25.3
"	"	"	1931	59.5	0.0	0.0	0.0	0.9	39.7	27.5	18.0	0.0	0.0	7.4	18.4
"	"	"	1932	....	0.0	0.0	0.0	2.3	1.9	4.8	3.8	1.5	0.7	8.0	8.4
Cheyenne	26	1930	1931	0.0	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	2.2	0.0	48.4
"	"	"	1932	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	1.4	3.7	0.0	44.0
Clark	3	1928	1929	0.0	0.0	2.4	10.4	0.0	0.0	0.3	2.3	0.0	4.5	0.2	41.6
"	40	1931	1932	0.0	0.0	0.3	0.6	0.0	0.0	0.0	0.3	0.0	3.9	0.0	30.3
"	41	"	"	0.0	0.6	0.0	1.9	1.6	0.0	0.0	0.0	0.5	1.7	0.0	33.8
Comanche	4	1928	1929	0.0	0.0	0.6	5.1	0.8	0.0	0.0	5.6	0.0	1.3	2.1	60.4
"	42	1931	1932	2.1	1.1	0.6	1.3	1.6	0.0	0.0	0.3	0.0	0.4	0.0	20.9
"	43	"	"	1.4	0.4	0.3	0.0	0.3	0.0	0.0	0.0	0.0	1.7	0.0	23.9
Edwards	27	1930	1931	5.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.5	0.0	0.0	55.6
"	"	"	1932	0.4	1.1	1.1	1.0	1.2	0.0	0.0	2.3	0.0	0.7	0.2	37.4
"	46	1931	"	5.7	0.4	0.0	0.5	0.9	0.4	1.5	2.4	0.7	0.0	0.5	31.8
"	47	"	"	0.0	0.8	0.3	3.3	0.3	0.0	0.0	2.5	0.7	3.6	0.0	48.3
"	48	"	"	0.0	0.0	0.6	..	3.0	0.0	0.0	1.1	0.0	8.5	0.3	64.4
"	49	"	"	..	1.8	0.7	1.9	8.9	0.0	0.5	5.5	0.0	3.4	0.0	61.0
Ford	5	1928	1929	0.0	0.0	3.0	3.6	0.8	0.0	0.0	1.4	0.0	0.8	1.8	73.3
"	44	1931	1932	1.5	0.0	3.3	1.3	1.7	0.0	1.6	0.0	1.3	2.5	0.0	42.9
"	45	"	"	0.0	0.0	0.3	0.4	0.3	0.0	0.0	3.0	0.9	2.3	0.0	16.3
Gove	18	1929	1930	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.0	2.5	0.6	0.0	31.6
"	"	"	1931	0.0	0.0	0.0	3.7	1.8	0.0	0.0	0.0	1.4	0.0	0.0	50.0
"	"	"	1932	0.0	1.1	2.0	3.1	1.1	0.0	0.0	1.1	0.0	7.9	0.0	51.8
Gray	6	1928	1929	0.0	0.0	1.2	1.4	0.4	0.0	0.0	1.8	0.0	0.8	0.0	60.3
"	7	"	"	0.0	0.9	3.0	2.4	0.0	0.0	0.0	2.7	0.0	0.0	0.7	68.3
"	50	1931	1932	0.0	0.0	3.4	1.5	1.0	0.0	0.0	0.6	0.3	1.2	1.0	35.4
Greeley	19	1929	1930	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	22.9
"	"	"	1931	19.4	0.0	1.0	2.0	0.0	0.0	7.1	2.1	1.4	1.2	0.8	54.3
"	"	"	1932	13.3	0.5	0.0	0.8	4.2	3.4	0.9	1.7	0.0	4.1	0.3	39.9
Hamilton	28	1930	1931	2.6	7.5	0.0	0.0	2.4	2.5	7.1	1.6	1.6	1.6	0.4	52.2
"	"	"	1932	5.4	1.7	..	0.0	0.3	1.3	0.0	0.3	0.0	2.8	0.3	15.4
Harper	20	1929	1930	0.0	0.0	0.0	0.0	4.2	1.0	0.5	0.7	0.6	1.1	0.0	34.3
"	"	"	1931	4.9	0.0	1.0	1.9	0.0	3.9	3.2	1.9	0.6	2.2	0.7	36.7
"	"	"	1932	5.4	0.0	0.3	0.5	0.6	0.0	0.0	2.9	0.5	6.2	0.0	71.4
Jewell	21	"	1930	0.0	0.0	0.4	0.0	0.3	0.0	0.0	0.0	0.3	0.0	0.0	26.9
"	"	"	1931	0.0	0.0	0.0	1.4	3.2	0.0	0.0	0.0	0.6	0.0	0.0	44.0
"	"	"	1932	0.0	0.0	0.5	4.0	1.2	0.0	0.3	0.3	0.2	0.9	0.0	51.9
Johnson	8	1928	1929	34.4	0.0	0.7	0.0	0.6	5.9	6.9	15.3	0.0	1.4	5.5	49.9
"	"	"	1930	7.5	0.0	0.0	0.0	0.0	0.9	1.0	4.5	0.0	0.0	2.4	11.4
"	"	"	1931	59.8	0.9	0.0	0.0	5.9	39.6	18.0	25.1	0.9	1.8	6.3	35.9

TABLE 1.—(Continued)

Source of col- lection	Collec- tion No.	Year		Percentage of heads bunted											
		Col- lected	Grown	Banner Berkeley	Hussar	Oro	Ridit	Turkey 1558A	Martin	White Odessa	Kooperatorka	Turkey x Bearded	Yogo	Regal	Kanred
<i>County</i>															
Johnson	8	1928	1932	31.0	1.1	0.8	0.7	0.6	5.6	9.7	24.5	1.1	0.3	1.9	45.9
Kiowa	29	1930	1931	3.1	0.0	0.0	0.0	0.0	0.0	0.7	1.9	0.0	0.0	0.0	61.4
"	"	"	1932	2.1	0.8	1.1	0.4	2.4	0.3	0.4	0.6	0.0	6.5	0.0	44.8
Lane	22	1929	1930	0.0	0.0	0.0	0.5	4.1	0.0	0.0	1.6	0.0	0.0	0.0	19.1
"	"	"	1931	0.0	0.0	0.0	0.9	5.8	0.0	0.0	2.4	0.7	0.8	0.0	54.4
"	"	"	1932	0.0	1.7	0.3	1.5	1.4	0.0	0.6	0.9	1.2	2.8	0.0	41.6
"	51	1931	"	0.5	1.0	0.0	0.9	1.3	0.0	0.0	1.9	1.0	3.5	0.4	60.8
"	52	"	"	0.4	0.0	0.0	1.3	.....	0.0	.....	0.0	.....	1.4	0.0	33.7
"	53	"	"	20.3	3.6	0.3	1.4	1.8	1.4	1.8	1.4	0.0	0.7	0.3	43.1
"	54	"	"	0.8	.....	0.7	0.4	0.6	0.0	0.0	1.0	1.3	6.8	0.0	65.8
Lincoln	30	1930	1931	0.0	0.0	0.0	2.2	0.0	0.0	0.0	3.9	0.0	2.7	0.0	67.8
"	"	"	1932	0.0	0.7	0.7	1.3	0.6	0.0	0.0	0.3	0.5	7.2	0.0	53.1
"	55	1931	"	0.0	0.0	0.0	2.1	3.0	0.0	0.0	1.4	0.0	3.5	0.0	68.2
"	56	"	"	6.3	2.6	8.7	2.1	3.6	1.0	0.4	1.0	1.2	7.4	.....	73.2
McPherson	9	1928	1929	0.0	0.0	3.1	0.0	0.6	0.0	0.0	1.8	0.0	5.9	1.2	85.5
Meade	10	"	"	0.0	0.0	1.8	1.5	0.2	0.0	0.0	1.5	0.0	3.4	2.3	77.8
"	23	1929	1930	0.0	7.4	0.0	0.6	5.1	1.7	1.1	0.0	0.0	3.4	0.0	45.1
"	"	"	1931	6.0	14.1	0.0	8.9	5.2	14.4	15.0	2.8	0.0	1.1	0.0	56.1
"	"	"	1932	0.0	0.0	0.3	1.7	0.5	0.4	0.0	0.0	0.0	2.2	0.0	61.8
Mitchell	57	1931	"	5.3	0.0	1.5	1.1	0.3	0.0	0.4	0.7	0.0	1.1	0.0	39.5
Ness	11	1928	1929	0.2	0.0	0.0	0.0	1.4	0.0	2.2	6.5	0.2	2.6	3.7	72.1
Norton	58	1931	1932	1.9	0.5	1.5	1.6	1.4	0.0	0.0	1.2	0.0	1.3	0.0	46.5
Pawnee	12	1928	1929	0.2	0.0	0.0	0.5	6.2	0.0	0.0	0.6	0.8	2.8	0.7	82.3
Pratt	14	"	"	0.0	0.0	0.0	0.0	14.4	0.0	0.0	1.6	0.0	0.0	0.0	53.4
"	59	1931	1932	0.0	2.4	0.0	5.5	0.3	0.5	0.0	0.3	0.0	2.2	0.0	44.4
Reno	13	1928	1929	0.0	0.0	0.9	0.0	6.6	0.0	0.0	0.0	0.0	3.0	0.0	60.5
"	31	1930	1931	0.0	1.1	0.0	0.0	2.8	0.0	0.0	0.0	0.0	1.5	0.0	62.7
"	"	"	1932	1.9	0.0	0.0	1.7	2.0	0.0	0.0	0.8	0.7	1.4	0.0	34.1
Rooks	32	"	1931	0.0	0.0	0.0	1.3	0.0	0.0	0.0	2.3	1.3	1.8	0.0	66.5
"	"	"	1932	0.5	3.1	0.5	0.5	0.6	0.0	0.0	2.2	0.0	3.3	0.7	40.6
Russell	64	1931	"	..	1.4	1.1	0.5	0.0	0.5	0.0	1.8	0.3	1.0	0.0	50.9
Scott	36	1930	1931	6.4	1.4	0.0	0.0	7.3	0.0	0.0	3.9	0.0	0.0	1.6	70.0
"	"	"	1932	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.3	1.0	0.0	30.8
Sedgwick	33	"	1931	0.0	0.0	0.0	0.0	3.5	0.0	0.0	0.0	0.0	0.0	0.0	60.9
"	"	"	1932	3.4	0.3	0.7	0.0	0.8	0.4	0.0	0.9	0.0	3.1	0.0	33.8
Sheridan	60	1931	"	0.0	0.4	0.3	0.9	1.7	0.0	0.0	1.0	0.7	1.9	0.0	39.7
"	61	"	"	0.0	0.0	1.2	5.2	0.0	0.0	0.0	1.0	1.3	2.7	0.0	33.8
Sherman	62	"	"	1.1	1.1	0.0	1.4	2.5	0.9	0.0	1.5	0.0	2.8	1.5	29.9
"	63	"	"	2.7	0.0	0.0	1.5	2.0	0.0	0.0	0.3	0.3	0.0	0.0	23.6
Smith	24	1929	1930	0.0	0.0	0.0	0.0	0.7	0.0	0.0	1.8	0.0	0.0	0.0	33.6
"	"	"	1931	1.2	0.0	0.0	1.2	2.3	0.0	0.0	3.1	0.6	1.9	0.0	51.5
"	34	1930	"	2.4	0.0	0.0	3.5	0.0	0.0	0.0	1.5	0.6	0.8	0.0	73.2
"	35	"	"	0.0	0.0	0.0	1.2	0.0	0.0	0.0	3.9	1.4	1.3	0.0	44.0
"	"	"	1932	0.0	0.0	0.3	0.7	1.9	0.0	0.0	0.0	0.6	0.4	0.0	56.7
Sumner	25	1929	1930	0.0	2.2	0.0	0.0	2.1	0.0	0.0	0.8	0.0	0.0	0.0	23.8
"	"	"	1931	0.0	0.0	0.0	3.3	1.6	0.0	0.0	4.0	0.0	0.0	0.0	56.3
"	"	"	1932	0.0	0.0	1.4	0.0	0.6	0.0	0.0	0.0	1.1	0.3	0.3	23.0
Thomas	15	1928	1929	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	2.1	1.4	41.4

interest to note the consistent reaction of Kanred, a commercial variety of considerable importance, which is highly susceptible to every collection made.

One may raise the question whether a bunt percentage, such as 7.4 in the case of Meade County No. 23 in a variety like Hussar is sufficiently high to differentiate this collection as a distinct form of the bunt organism. This variety has zero or trace to 14+ per cent of bunt from all other collections. The fact that Meade County No. 23 collection is a distinct form with high infection potentialities is shown in tables 3 and 4. When the variety Hussar was successively inoculated with this collection of bunt for 3 years, the resulting infections were 7.4 per cent in 1930, 47.2 per cent in 1931, and 16.6 per cent bunt in 1932. The striking reaction of this collection of smut when used to inoculate the differential varieties in 1932, after it had been increased on Hussar for two years, is shown in table 4. This shows plainly how a new form may be separated and increased on a variety that is generally regarded as highly resistant. This process was observed in other instances during the course of these investigations. In a few cases, however, such apparent increases proved to be mere fluctuations. The subject of reinoculations is more fully discussed on page 1216.

A summary of studies of the bunt collections is given in table 2 where the collections are grouped into physiologic forms according to their reaction on the differential varieties. Where a collection of bunt has been studied for 2 or more years, an average of the smut percentages for each variety has been made.

The physiologic-form numbers that have been assigned in this paper have no direct connection with the numbers assigned by other investigators of the bunt problem, since the differential varieties used are not necessarily the same. The writer realizes that some of the physiologic forms here recognized probably are questionable because the percentages of bunt are rather low, but since it was amply demonstrated that they differed from known forms, numbers have been assigned to readily distinguish them. It became apparent in these studies that a definite classification of all collections of bunt is not possible, but an attempt has been made to group the collections that have similar pathogenic tendencies on the standard varieties of wheat. In studies of this kind there always is a question in regard to form differentiation; *i.e.*, whether or not some physiologic forms have not been separated on too narrow a margin. This may be the case in some instances in these studies. However, if the data are studied statistically over a period of years, it is very probable that they will be found to be significant.

It is well known that bunt percentages differ from year to year because of environmental conditions. This was demonstrated in these studies by

**TABLE 2.**—*The average percentage of bunt obtained on a selected group of wheat varieties, and the physiologic form numbers assigned to the bunt collections, Manhattan, Kansas*

Source of smut	Collection No.	Year collected	Number of years tested	Average percentage of smutted heads											
				Banner Berkeley	Hussar	Oro	Ridit	Turkey Sel. 1558A	Martin	White Odessa	Kooperatoroka	Turkey x Bearded	Yogo	Regal	Kanred
County				Physiologic Form 1											
Barton	16	1929	3	0.2	0.0	0.5	0.8	0.7	0.0	0.0	0.4	0.8	1.1	0.0	37.9
"	37	1931	1	0.0	0.0	0.7	0.4	1.2	0.0	0.0	0.8	0.0	2.1	0.0	27.5
"	38	"	1	0.0	0.0	0.4	1.1	2.4	0.0	0.0	0.7	0.9	0.6	0.2	12.7
"	39	"	1	1.2	0.0	0.0	0.4	0.4	0.0	0.0	0.4	0.0	2.8	0.0	61.7
Cheyenne	26	1930	2	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.7	3.0	0.0	46.2
Clark	40	1931	1	0.0	0.0	0.3	0.6	0.0	0.0	0.0	0.3	0.0	3.9	0.0	30.3
"	41	"	1	0.0	0.6	0.0	1.9	1.6	0.0	0.0	0.0	0.5	1.7	0.0	33.8
Comanche	42	1931	1	2.1	1.1	0.6	1.3	1.6	0.0	0.0	0.3	0.0	0.4	0.0	20.9
"	43	"	1	1.4	0.4	0.3	0.0	0.3	0.0	0.0	0.0	0.0	1.7	0.0	23.9
Edwards	27	1930	2	2.7	0.5	0.5	0.5	0.6	0.2	0.0	1.1	0.2	0.4	0.1	46.5
"	46	1931	1	5.7	0.4	0.0	0.5	0.9	0.4	1.5	2.4	0.7	0.0	0.5	31.8
"	47	"	1	0.0	0.8	0.3	3.3	0.2	0.0	0.0	2.5	0.7	3.6	0.0	48.3
"	48	"	1	0.0	0.0	0.6		3.0	0.0	0.0	1.1	0.0	8.5	0.3	64.4
Ford	44	1931	1	1.5	0.0	3.3	1.3	1.7	0.0	1.6	0.0	1.3	2.5	0.0	42.9
"	45	"	1	0.0	0.0	0.3	0.4	0.3	0.0	0.0	3.0	0.9	2.3	0.0	16.3
"	5	1928 <sup>a</sup>	1	0.0	0.0	3.0	3.6	0.8	0.0	0.0	1.4	0.0	0.8	1.8	73.3
Gove	18	1929	3	0.0	0.4	0.7	2.3	1.0	0.0	0.3	0.7	1.3	2.9	0.0	44.4
Gray	6	1928 <sup>a</sup>	1	0.0	0.0	1.2	1.4	0.4	0.0	0.0	1.8	0.0	0.8	0.0	60.3
"	7	" <sup>a</sup>	1	0.0	0.9	3.0	2.4	0.0	0.0	0.0	2.7	0.0	0.0	0.7	68.3
"	50	1931	1		0.0	3.4	1.5	1.0	0.0	0.0	0.6	0.3	1.2	1.0	35.4
Harper	20	1929	3	3.4	0.0	0.4	0.8	1.6	1.6	1.2	1.8	0.5	3.2	0.2	47.4
Jewell	21	1929	3	0.0	0.0	0.3	1.8	1.9	0.0	0.1	0.1	0.4	0.3	0.0	40.9
Kiowa	29	1930	2	2.6	0.4	0.6	0.2	1.2	0.2	0.6	1.2	0.0	3.3	0.0	51.1
Lane	54	1931	1	0.8		0.7	0.4	0.6	0.0	0.0	1.0	1.3	6.8	0.0	65.8
"	51	"	1	0.5	1.0	0.0	0.9	1.3	0.0	0.0	1.9	1.0	3.5	0.4	60.8
"	52	"	1	0.4	0.0	0.0	1.3		0.0		0.0		1.4	0.0	33.7
Lincoln	30	1930	2	0.0	0.4	0.3	1.8	0.3	0.0	0.0	2.1	0.2	5.0	0.0	60.5
"	55	1931	1	0.0	0.0	0.0	2.1	3.0	0.0	0.0	1.4	0.0	3.5	0.0	68.2
McPherson	9	1928 <sup>a</sup>	1	0.0	0.0	3.1	0.0	0.6	0.0	0.0	1.8	0.0	5.9	1.2	85.5
Meade	10	1928 <sup>a</sup>	1	0.0	0.0	1.8	1.5	0.2	0.0	0.0	1.5	0.0	3.4	2.3	77.8
Mitchell	57	1931	1	5.3	0.0	1.5	1.1	0.3	0.0	0.4	0.7	0.0	1.1	0.0	39.5

TABLE 2.—(Continued)

Source of smut	Collection No.	Year collected	Number of years tested	Average percentage of smutted heads											
				Banner Berkeley Hussar	Oro	Ridit	Turkey Sel. 1558A	Martin	White Odessa	Kooperatorka	Turkey x Bearded	Yogo	Regal	Kanred	
County				Physiologic Form 1—(Cont.)											
Ness	11	1928 <sup>a</sup>	1	0.2	0.0	0.0	0.0	1.4	0.0	2.2	6.5	0.2	2.6	3.7	72.1
Norton	58	1931	1	1.9	0.5	1.5	1.6	1.4	0.0	0.0	1.2	0.0	1.3	0.0	46.5
Reno	31	1930	2	1.0	0.6	0.0	0.9	2.4	0.0	0.0	0.4	0.3	1.4	0.0	48.4
Rooks	32	1930	2	0.3	1.6	0.2	0.9	0.3	0.0	0.0	2.3	0.7	2.6	0.4	53.5
Russell	64	1931	1		1.4	1.1	0.5	0.0	0.5	0.0	1.8	0.3	0.0	0.0	50.9
Sheridan	60	1931	1	0.0	0.4	0.3	0.9	1.7	0.0	0.0	1.0	0.7	1.9	0.0	39.7
Sherman	62	1931	1	1.1	1.1	0.0	1.4	2.5	0.9	0.0	1.5	0.0	2.8	1.5	29.9
"	63	"	1	2.7	0.0	0.0	1.5	2.0	0.0	0.0	0.3	0.3	0.0	0.0	23.6
Smith	24	1929	2	0.6	0.0	0.0	0.6	1.5	0.0	0.0	2.4	0.3	0.9	0.0	42.5
"	34	1930	1	2.4	0.0	0.0	3.5	0.0		0.0	1.5	0.6	0.8	0.0	73.2
"	35	"	2	0.0	0.0	0.2	0.9	1.0	0.0	0.0	1.9	1.0	0.9	0.0	50.4
Sedgwick	33	1930	2	1.7	0.2	0.4	0.0	2.1	0.2	0.0	0.4	0.0	1.6	0.0	47.3
Sumner	25	1929	3	0.0	0.7	0.5	1.1	1.5	0.0	0.0	1.6	0.4	0.1	0.1	34.3
Thomas	15	1928 <sup>a</sup>	1	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	2.1	1.4	41.4
				Physiologic Form 2											
Brown	17	1929	3	27.5 <sup>b</sup>	0.0	0.0	0.0	1.4	14.6	11.5	8.7	0.8	0.2	6.0	17.4
Johnson	8	1928	4	33.2	0.5	3.8	0.2	1.8	12.8	8.9	17.4	0.5	0.9	4.0	35.6
				Physiologic Form 3											
Hamilton	28	1930	2	4.0	4.6	0.0	0.0	1.4	1.9	3.6	0.9	0.8	2.2	0.3	33.8
Meade	23	1929	3	2.0	7.2	0.1	3.7	3.9	5.5	5.4	0.9	0.0	2.2	0.0	54.4
				Physiologic Form 4											
Edwards	49	1931	1		1.8	0.7	1.9	8.9	0.0	0.5	5.5	0.0	3.4	0.0	61.0
Lane	22	1929	3	0.0	0.7	0.1	0.9	3.7	0.0	0.2	1.6	0.6	1.2	0.0	38.4
Pawnee	12	1928 <sup>a</sup>	1	0.2	0.0	0.0	0.5	6.2	0.0	0.0	0.6	0.8	3.8	0.7	82.3
Pratt	14	1928 <sup>a</sup>	1	0.0	0.0	0.0	0.0	14.4	0.0	0.0	1.6	0.0	0.0	0.0	53.4
Reno	13	1928 <sup>a</sup>	1	0.0	0.0	0.9	0.0	6.6	0.0	0.0	0.0	0.0	3.0	0.0	60.5
Scott	36	1930	2	3.2	0.7	0.0	0.0	4.2	0.0	0.0	2.0	0.2	0.5	0.8	50.4

TABLE 2.—(Continued)

Source of smut	Collection No.	Year collected	Number of years tested	Average percentage of smutted heads											
				Banner Berkeley	Hussar	Oro	Ridit	Turkey Sel. 1558A	Martin	White Odeisa	Kooperatoroka	Turkey x Bearded	Yogo	Regal	Kanred
County				Physiologic Form 5											
Barton	1	1928 <sup>a</sup>	1	0.0	0.0	3.1	5.7	0.2	0.0	0.2	0.4	0.0	2.8	0.4	61.9
"	2	1928 <sup>a</sup>	1	0.0	0.0	0.6	11.2	0.0	0.0	0.0	1.5	0.0	2.2	2.5	59.4
Clark	3	1928 <sup>a</sup>	1	0.0	0.0	2.4	10.4	0.0	0.0	0.3	2.3	0.0	4.5	0.2	41.6
Comanche	4	1928 <sup>a</sup>	1	0.0	0.0	0.6	5.1	0.8	0.0	0.0	5.6	0.0	1.3	2.1	60.4
Pratt	59	1931	1	0.0	2.4	0.0	5.5	0.3	0.5	0.0	0.3	0.0	2.2	0.0	44.4
Sheridan	61	1931	1	0.0	0.0	1.2	5.2	0.0	0.0	0.0	1.0	1.3	2.7	0.0	33.8
				Physiologic Form 6											
Lincoln	56	1931	1	6.3	2.6	8.7	2.1	3.6	1.0	0.4	1.0	1.2	7.4		73.2
				Physiologic Form 7											
Greeley	19	1929	3	10.9	0.2	0.3	0.9	1.4	1.2	2.7	1.5	0.5	1.8	0.4	39.1
Lane	53	1931	1	20.3	3.6	0.3	1.4	1.8	1.4	1.8	1.4	0.0	0.7	0.3	43.1

<sup>a</sup> Tested only one year. Through error, these bunt collections were discarded.

<sup>b</sup> Average for 2 years, 1930–1931, as results for Banner Berkeley, 1932, were lost.

the fact that the bunt percentages were higher in 1929 and 1931 than in 1930 and 1932. It also is evident that certain bunt collections may undergo changes in physiologic-form content from year to year, even though carefully handled. For this reason a collection of bunt may attack a differential variety heavily one year and less so the following year, thereby lowering the average percentage of infection over a series of years. This is evident from table 1.

The data in table 1 indicate that the collections fall rather naturally into 7 groups, according to varietal reactions. Some groups contained collections from many sources, while others contained only a few. After the data were averaged and arranged by forms, as in table 2, it became clear that each form had a few outstanding characteristics by which it could be quickly and readily recognized. These characteristics are as follows:

Physiologic form 1 is characterized by low or zero infection on all varieties except Kanred. Forty-seven collections of bunt are included in this physiologic form.

Physiologic form 2 is characterized by high infection on Banner,

Berkeley and moderately high infection on Martin, White Odessa, and Kooperatorka. Two bunt collections are included in this physiologic form.

Physiologic form 3 is characterized by comparatively high infection on Hussar and a moderate amount of infection on Martin and White Odessa. Two bunt collections are included in this form.

Physiologic form 4 is characterized by moderately high infection on Turkey. This form includes 6 bunt collections.

Physiologic form 5 is characterized by moderately high infection on Redit and includes 6 bunt collections.

Physiologic form 6 is characterized by moderately high infection on Oro, while Hussar, Martin, and White Odessa are resistant. Only one bunt collection is included in this form.

Physiologic form 7 is characterized by high infection on Banner Berkeley and low infection on Martin, in contrast to physiologic form 2, to which Martin is more susceptible. Two bunt collections are included in this form.

On the basis of the grouping in table 2, a dichotomous key has been prepared for the identification of the physiologic forms occurring in the Kansas collections of bunt tested in the seasons of 1929 to 1932, inclusive. Seven forms are separated by differences in pathogenicity on Banner Berkeley, Hussar, Oro, Redit, Turkey, and Martin. Regal and Kooperatorka have possibilities as differential varieties and could be used in the identification of certain forms. The six varieties used as differentials adequately separate the forms in these investigations. Yogo and Turkey × Bearded show some resistance to all Kansas collections of bunt tested. The latter especially seems to be the most resistant of any of the varieties grown.

#### KEY FOR THE IDENTIFICATION OF PHYSIOLOGIC FORMS OF BUNT IN KANSAS

Banner Berkeley resistant	
Hussar highly resistant	
Oro highly resistant	<i>Form</i>
Redit highly resistant	<i>No.</i>
Turkey Sel. highly resistant	1
Turkey Sel. moderately susceptible	4
Redit moderately susceptible	5
Oro moderately susceptible	6
Hussar moderately susceptible	3
Banner Berkeley susceptible	
Martin resistant	7
Martin susceptible	2

DISTRIBUTION AND PREVALENCE OF PHYSIOLOGIC FORMS OF  
BUNT IN KANSAS

The distribution of the physiologic forms of bunt in Kansas, based on the collections tested, is shown in figure 1 (lower map). The collections were made in those counties having county agricultural agents and are in areas where wheat production is an important industry. Not all the State, however, is represented, as it was necessary to make the collections where bunt was readily available and through the aid of the county agents and extension specialists.

It will be observed that form 1 is apparently very widely distributed over the hard red winter-wheat belt of the State and is by far the most common form. Form 2 is confined to the soft red winter-wheat sections of northeastern Kansas. Further studies are needed to show just how common or widely distributed it is. Form 3 appears in 2 counties in southwestern Kansas, while forms 4 and 5 are found distributed over the south-central portion of the State. Form 6 was found only in Lincoln county in the central part of the State, and form 7 occurs in 2 of the west-central counties.

REINOCULATING VARIETIES OF WHEAT WITH THEIR OWN BUNT

In connection with a study of the physiologic forms of bunt, an attempt was made to ascertain how these forms might have originated and how they might have increased. Dillon Weston holds that resistant varieties "break down" in their resistance to bunt (21). This same investigator and Bressman (2) have been able to increase the percentage of bunt in Ridit by inoculating seed of this variety with bunt obtained from smutted Ridit heads.

Reinoculation experiments were begun at the Kansas Agricultural Experiment Station in 1929 in which such resistant varieties as Hussar, White Odessa, Yogo, Turkey  $\times$  Bearded, Kooperatorka, Martin, and Ridit were used. In cases where traces of bunt occurred on these varieties, as grown in the bunt nursery, the bunted heads were saved and used as inoculum in the reinoculation of the same variety the following year. By this method it should be possible to determine whether this bunt is especially virulent on a variety regarded as resistant. If so, this form of bunt should increase in abundance on that variety in succeeding years. These studies showed that in some instances a strain of smut increased, while in others it seemed to become innocuous and finally disappeared. The results of these reinoculations are given in tables 3 and 4. It will be noted that by reinoculating certain varieties the smut percentages were increased during the course of the studies. Significant increases over 1930 infections are to be noted in 10 cases out of 32 (Table 3). In one case (Turkey



TABLE 3.—*Varieties showing small percentages of bunt infection in 1929, which were reinoculated with their own smut in 1930-1932. Manhattan, Kansas*

1930 Culture No.	Varietal source of bunt	County source of bunt 1929	Percentage of bunt when reinoculated		
			1930	1931	1932(a)
107	Turkey × Bearded .....	Barton	2.5	1.8	.....
112	Kooperatorka .....	"	1.0	0.3	.....
116	Martin .....	Brown	2.2	24.9	.....
117	White Odessa .....	"	2.5	23.1	.....
122	Banner Berkeley .....	"	23.1	57.9	27.9
123	Regal .....	"	2.5	12.3	.....
129	White Odessa .....	Gove	0.9	0.0	.....
131	Turkey × Bearded .....	"	2.5	0.0	.....
132	Yogo .....	"	0.6	1.2	.....
136	Kooperatorka .....	"	1.0	0.0	.....
148	Kooperatorka .....	Greeley	0.8	0.0	.....
149	Turkey 1558A .....	Harper	4.2	3.6	.....
152	Martin .....	"	1.0	8.0	.....
155	Turkey × Bearded .....	"	0.6	0.8	.....
156	Yogo .....	"	1.1	0.0	.....
166	Oro .....	Jewell	0.4	0.0	.....
167	Turkey × Bearded .....	"	0.3	2.5	0.0
177	White Odessa .....	Johnson	1.0	11.6	.....
182	Banner Berkeley .....	"	7.5	55.2	.....
185	Turkey 1558A .....	Lane	4.1	0.0	.....
193	Ridit .....	"	0.5	2.4	3.8
196	Kooperatorka .....	"	1.6	1.4	0.0
197	Turkey 1558A .....	Meade	5.1	6.0	.....
199	Hussar .....	"	7.4	47.2	16.6
200	Martin .....	"	1.7	23.5	.....
201	White Odessa .....	"	1.1	37.9	15.5
204	Yogo .....	"	3.4	0.9	.....
205	Ridit .....	"	0.6	14.8	.....
220	Kooperatorka .....	Smith	1.8	2.3	.....
221	Turkey 1558A .....	Sumner	2.1	3.4	.....
223	Hussar .....	"	2.2	2.3	.....
232	Kooperatorka .....	"	0.8	2.4	.....

(a) The bunt percentages were much lower for even the most susceptible varieties in 1932, so that the percentages in this column may be considered significant.

Sel. culture No. 185, Lane County smut) the percentage was significantly reduced. This is not unexpected. There is the possibility that the smut obtained in the variety came from a plant that was not typical of the variety. Naturally, one would not expect this bunt to increase on the typical variety if the smut were used the following year. It may also happen that the smut present on a resistant variety consists of one or only

TABLE 4.—*Reinoculating a strain of bunt on a normally resistant variety of wheat, thereby increasing the percentage of infection*

Source of bunt Kans., 1929	Bunt collec- tion number	Year of Back- inoculation	Percentage of bunted heads											
			Banner Berkeley	Hussar	Oro	Ridit	Turkey	Martin	White Odessa	Kooperatoroka	Turkey x Bearded	Yogo	Regal	Kanred
County														
Brown	17	1930	23.1	0.0	0.0	0.0	1.0	2.2	2.1	4.3	1.0	0.0	2.5	25.3
Reinoculated	17a	1931	57.9											
on Banner Berkeley	17a	1932	27.9	0.4	0.3	1.4	0.5	3.0	7.2	5.0	0.0	2.2	3.3	6.0
Meade	23	1930	0.0	7.4	0.0	0.6	5.1	1.7	1.1	0.0	0.0	3.4	0.0	45.1
Reinoculated	23a	1931	47.2											
on Hussar	23a	1932	69.4	16.6	0.7	1.4	1.6	15.5	10.5	17.7	2.9	2.7	6.7	19.5
Meade	23	1930	0.0	7.4	0.0	0.6	5.1	1.7	1.1	0.0	0.0	3.4	0.0	45.1
Reinoculated	23b	1931							37.9					
on White Odessa	23b	1932	43.8	14.6	2.8	0.0	1.9	3.1	15.5	14.1	0.0	4.5	6.4	35.4

a part of a single bunted head, perhaps on a late, small, poorly developed tiller. The smut dosage has a direct bearing on the percentage of bunt produced, and, if the amount of inoculum has to be spread too thinly over the seed for a row five feet in length, there may be no smut in the crop. This may be the explanation for the low infections of bunt in cultures 129, 131, 136, and 148 in 1930 (Table 3).

The varieties listed in table 3 are, generally speaking, resistant to bunt in Kansas, especially when they are compared with Kanred and Blackhull. The results of the reinoculation studies in 1932 are given for a 3-year period in 6 of the 32 cultures used in these studies in 1930 and 1931. These 6 cases included 3 that had shown rather high smut percentages and 3 that were low. No attempt has been made to see how many new physiologic forms might be demonstrated by this "straining method" or building up of the inoculum on resistant varieties. Banner Berkeley culture No. 122, Hussar culture No. 199, and White Odessa culture No. 201 gave high percentages of bunt. The results seem especially interesting in the case of Hussar, in which the bunt has increased from 7.4 to 47.2 per cent in one year, and in White Odessa culture No. 201, in which the bunt increased from 1.1 to 37.9 per cent. In contrast to these, Ridit culture No. 193 had 0.5 per cent in 1930 and 3.8 per cent in 1932, a very small increase of doubtful significance. A more clear-cut demonstration of what may occur in reinoculation studies is exhibited in table 4. Three collections of bunt

were made in 1929 and used to inoculate differential varieties with results as shown for 1930. In 1931 Banner Berkeley, Hussar, and White Odessa were each reinoculated with their own smut. The smut produced in 1931 on those 3 varieties was again used to inoculate the entire series of differential varieties in 1932.

Banner Berkeley, Hussar, and White Odessa are considered resistant varieties, at least to the majority of bunt collections of Kansas. Obviously, however, collections of bunt do occur that contain forms that attack these varieties. It also is clear that the percentages of infection may increase over a period of years if those forms are allowed to develop on the susceptible host variety. The season of 1932 was not favorable for heavy bunt infection, which explains why the percentages dropped from the high points of 1931 to lower percentages in 1932. Hussar had 7.4 per cent bunt in 1930, which resulted from inoculum applied from bunt collected in Meade County in 1929. This bunt from Hussar was reinoculated on Hussar and gave 47.2 per cent of smut in 1931. In 1932 this smut was applied not only to Hussar, but also to the other standard varieties. By comparing the reactions of the standard varieties to bunt in 1930 and 1932, it will be seen that Banner Berkeley, Hussar, Martin, White Odessa, Kooperatorka, and Regal show increased susceptibility.

Striking differences also may be noted in the reaction of White Odessa to the same collection of bunt. The same procedure of reinoculating White Odessa with its own bunt resulted in marked increases the following year on Banner Berkeley, Hussar, Kooperatorka, and Regal. These results fully justify the conclusion that the occurrence of a small percentage of bunt, such as 7.4 or even 1.1, on certain resistant varieties may be due to the presence of a distinct physiologic form occurring as a slight mixture in the inoculum.

On the other hand, it also is true that the appearance of small quantities of bunt on a resistant variety may not indicate the presence of a virulent physiologic form. Turkey  $\times$  Bearded had 0.3 per cent infection from culture No. 167 in 1930 and none in 1932. Kooperatorka had 1.6 per cent bunt from culture No. 196 in 1930 and none in 1932.

The results given in tables 3 and 4 leave little or no question that physiologic forms of smut may be built up in this manner. This makes it appear that a resistant variety may become susceptible and physiologic forms may be increased and spread by the introduction and increase of a new variety of wheat in a locality.

#### CULTURAL STUDIES OF PHYSIOLOGIC FORMS OF BUNT

It is possible to learn about the characteristics of physiologic forms of smut in several ways; namely, studies of cultural characteristics on arti-

ficial media, pathogenicity, physico-chemical properties, and, to a limited extent, morphologic differences, although the latter are less reliable. Since there are outstanding differences in the physiologic forms of *Tilletia laevis* as to their reaction on certain varieties of wheat, it seemed desirable to study their growth habits on artificial media.

Without attempting to record the details of the culture preparations, isolations of spores, and general technique, it will suffice to say that plain agar, soil-extract agar, and wheat (grain)-extract agar are satisfactory for the germination of chlamydospores of *Tilletia laevis* and for the growth of colonies of the fungus. Potato-sucrose agar (4 per cent) and oatmeal-dextrose (3 per cent) agar were found to be the best media. Five physiologic forms were found to show some differences in color, type of margin, topography, surface growth, and consistency of the colony (Fig. 2). They were selected on the basis of showing cultural growth differences. These forms are strikingly different also in the range of wheat varieties that they attack.

The cultures were made by isolating colonies of germinating chlamydospores. Cultures from single primary sporidia were not obtained, since it was difficult to make them grow after they were isolated. This is not the case with the sorghum kernel smut, *Sphacelotheca sorghi*, and some of the other smuts. The cultures described herein, therefore, are not so reliable for cultural study as those obtained from single sporidia, because of the phenomenon of heterothallism. However, as far as could be noted, the cultures obtained in this manner were quite consistent in their characteristics, even after several transfers were made. It was felt, however, that not a great deal of emphasis should be laid on the results obtained in the study of the bunt organism on artificial media, at least in so far as separating physiologic forms is concerned.

The studies soon revealed that *Tilletia laevis* develops very slowly, and the colonies, therefore, were grown in Erlenmeyer flasks to avoid rapid drying. The cultures were grown at temperatures ranging from 17° to 20° C. for a period of 2 months. The 5 physiologic forms, as grown on potato-sucrose agar, are described in table 5.

It was not possible to secure satisfactory cultures of all of the 7 forms; therefore, the record is given for only the 5 forms that were obtained. The cultures were separated by their growth and color differences. It was apparent, that the several forms cultured did not always produce "typical growth" characteristics each time they were grown from chlamydospores. This is not surprising in view of the recent studies by Flor (7), who obtained great variation, even among monosporidial cultures within physiologic forms. Also, the importance that should be attached to the cultural studies of bunt has been questioned by this same writer (7).

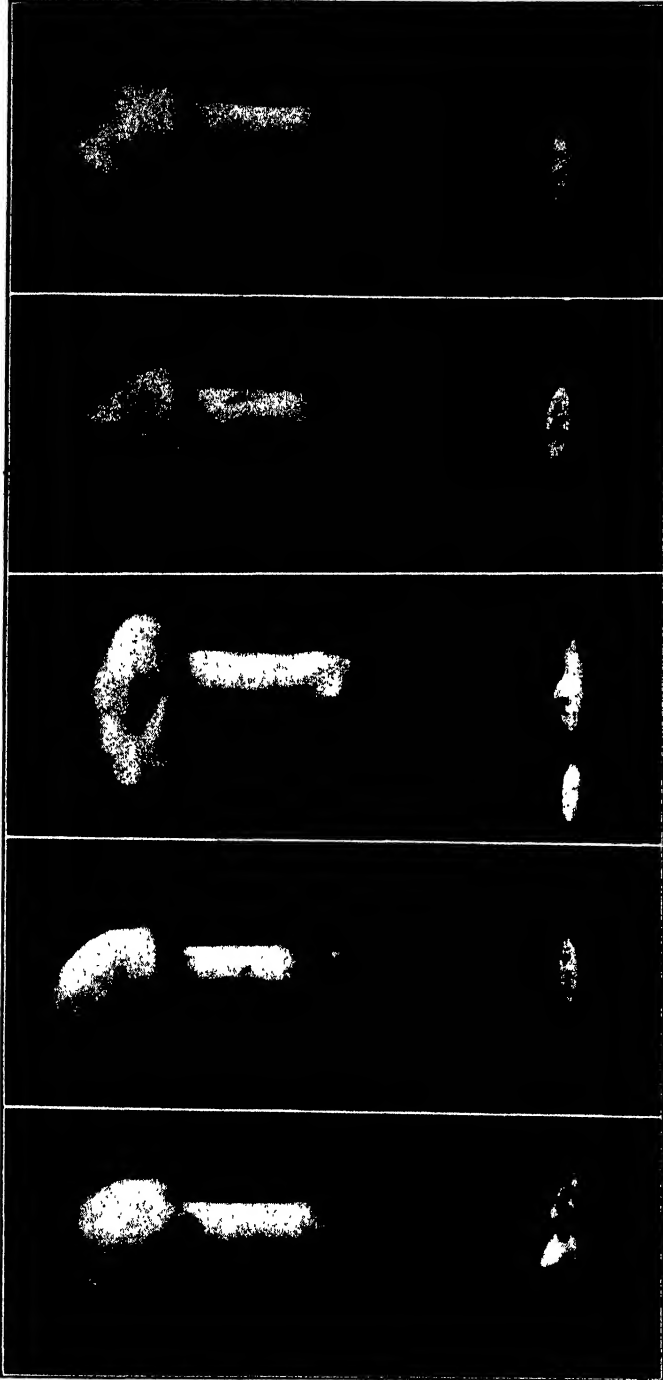


FIG. 2. Physiologic forms 1 to 5, of *Tilletia laevis*. Their cultural characteristics are described in table 5.

TABLE 5.—*Cultural characteristics of Tilletia laevis two months after planting. May 13, 1931*

Phy- lo- gic form	County source of smut	Year col- lected	Diam. of culture in mm.	Color	Consistency	Colony characters	Color of medium
1	Greeley	1929	15 × 10	Greyish.	Plastery to cottony.	Raised 10 mm.; margin very ir- regular; no exudate droplets; sporidial discharge; circular fur- rows around cottony mound.	Unchanged.
2	Johnson	1928	23 × 20	Bluish-white, few white cottony knobs.	Velvety.	Flat, except for few scattered knobs; margin irregular and defi- nite; no sporidial discharge; no exudate droplets; surface furrowed.	Slightly brown throughout.
3	Meade	1929	25	White.	Velvety with cottony margins.	Raised 6 mm.; margin irregular and definite; irregular mounds at center; sporidial discharge; few exudate droplets light brown; sur- face irregular and furrowed.	Slightly brown around colony.
4	Lane	1929	21	Greyish.	Cottony.	Flat, except for knobs bearing exudate droplets at center; colony nearly circular; margin irregular and definite; surface slightly fur- rowed.	Deep brown throughout.
5	Barton	1928	26	Pure white.	Velvety with cottony margins.	Raised 4 mm.; few plaster-like, white knobs at the center; margin irregular and definite; no sporidial discharge; few exudate droplets; surface of colony slightly furrowed.	Unchanged.

## A PROGRAM OF BREEDING FOR BUNT RESISTANCE IN WINTER WHEAT

The difficulties, due to the existence of physiologic forms, in breeding for bunt resistance, have been mentioned herèin and elsewhere (2). The task should not be considered as hopeless, however. The fact that there are some resistant varieties or selections in each of the groups of wheat, *i.e.*, in the 42-, the 28-, and the 14-chromosome groups, indicates that resistance is not specifically correlated with any gross morphologic type. This allows considerable liberty in the selection of resistant parental stock and greatly increases the chances of success. It should be possible, therefore, to secure a commercially adapted winter wheat for Kansas that is resistant to bunt. This should be possible by either selection or hybridization, but the possibilities of both methods should be fully explored. The promising resistant selections of Turkey wheat recently made in Nebraska (11) well illustrate the possibilities in that direction. A cooperative program for breeding for bunt resistance is under way at the Kansas Agricultural Experiment Station and some promising hybrid selections have been made, especially from the cross Oro  $\times$  Tenmarq.

The greater the range of the bunt collection to which the wheat selections or hybrids are subjected, the more dependable the final results will be. Even if tested to a number of forms of bunt, however, the resistance of a variety will probably be maintained only for a relatively short period of years. The greater the area over which the variety is to be grown, the greater the likelihood of encountering a physiologic form or forms that will attack the new variety. There also is the possibility that forms may be introduced from other states or counties. This problem must be faced by the pathologist and plant breeder, who should be cautious about making positive statements regarding the resistance of new varieties. There will then be fewer disappointments on the part of the research worker, and less criticism from administrators and from farmer taxpayers who support research in plant breeding and plant pathology. It is not likely that each new physiologic form that may be encountered will seriously change the picture. However, existing physiologic forms will gradually find their way into the region where a newly produced variety is grown and possibly alter the situation. How fast this may take place is a question needing further study. It should be recognized, however, that each new physiologic form discovered, or produced by hybridization or mutation, does not necessarily complicate the problem of breeding for resistance, since it is known that the resistance to certain groups of physiologic forms is inherited in a comparatively simple manner.

In a program of breeding for bunt resistance, the testing of the selections, varieties, or hybrids should consist of inoculating the seed with as many physiologic forms or collections of bunt as can be secured in the State

or region in which the varieties may be grown. This is not a new suggestion, but the author wishes to emphasize its importance. Later, the variety, selection, or hybrid may be planted in a series of uniform bunt nurseries where it will be inoculated with many forms of bunt and grown under varying environmental conditions. This method of testing represents more nearly what the variety is likely to encounter under conditions of wide distribution. New collections of bunt should be added to the forms already in use as the variety testing progresses. With this procedure, the actual reaction to bunt is more likely to be learned.

It is not a simple matter to determine the resistant parents to be used in crosses, because the literature shows that numerous varieties that at one time were regarded as resistant to bunt are no longer resistant to all forms of bunt (2, 10). This greatly limits the choice of varieties useful in hybridization. The truth of this is realized when one considers that varieties such as White Odessa, Albit, Regal, Martin, Hussar, Banner Berkeley, Oro, and Ridit, which have been called resistant or immune, are now known to be susceptible to one or more physiologic forms of the bunt organism as shown in these studies and by Bressman (2).

#### SUMMARY

Increasingly severe outbreaks of wheat bunt in Kansas in recent years indicate the presence of virulent strains of the bunt organism.

Tests were conducted in the bunt nursery of the Department of Botany at Manhattan, Kansas, over a 4-year period to determine the reaction of 12 selected varieties of winter wheat to many collections of bunt from various counties in the important wheat-growing sections of the State.

All collections of Kansas bunt were found on microscopic examination to belong to the species, *Tilletia laevis*.

Distinct differences in virulence among the bunt collections were manifested on the varieties Turkey (C.I. 1558A), Martin (C.I. 4463), Hussar (C.I. 4843), White Odessa (C.I. 4655), Oro (C.I. 8220), Ridit (C.I. 6703), and Banner Berkeley (C. I. 7362).

The collections of bunt have been grouped according to their types of virulence on these varieties into 7 physiologic forms. A dichotomous key for use in the separation of forms is presented in the text.

The varieties Turkey × Bearded (C.I. 8243) and Yogo (C.I. 8033) show resistance to all collections of Kansas bunt so far tested. Martin (C.I. 4463), Ridit (C.I. 6703), Hussar (C.I. 4843), White Odessa (C.I. 4655), Banner Berkeley (C.I. 7362), Regal (C.I. 7364), Oro (C.I. 8220), and Kooperatorka (C.I. 8861) show considerable resistance to most collections of bunt in Kansas, but the majority are known to be somewhat susceptible to certain forms in other parts of the United States.



A map is included showing the distribution of the 7 physiologic forms of *Tilletia laevis* in Kansas. Form 1 is the most widely distributed.

Reinoculating bunt on resistant varieties of wheat that showed small percentages of bunt the previous season resulted in an increased infection in 10 cases out of 32. Very decided increases in infection on certain resistant varieties suggest that new forms may have developed by segregation following hybridization.

Cultural studies have shown that plain agar, soil-extract agar, and wheat (grain)-extract agar are satisfactory for the germination of chlamydospores of *Tilletia laevis* and for the initiation of colony growth.

The best media for showing cultural differences among physiologic forms of bunt were found to be potato-sucrose (4 per cent) and oatmeal-dextrose (3 per cent) agar. Consistent and distinct differences in cultural characteristics were shown to exist among representative bunt collections of five physiologic forms.

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# TREATMENT OF SWEET-POTATO PLANTS FOR THE CONTROL OF BLACK ROT

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Sweet potatoes constitute one of the most important commercial crops in Mississippi. In 1932 the total production for the State was in excess of 7,900,000 bushels. Only Georgia, Alabama, and North Carolina exceeded this amount for that year. Now, with so large an acreage of land diverted from cotton to the growing of other crops, it is probable that a still greater production may eventuate in the immediate future.

A long and continued cropping of sweet potatoes without crop rotation and, at least in the first years of the industry, without much attention to sanitary measures to prevent disease dissemination, has resulted in a large amount of "disease-sick soil," that is, soil containing great numbers of sweet-potato pathogens.

Sweet-potato black rot, caused by the fungus, *Ceratostomella fimbriata* (E. & H.) Elliott, is one of the worst diseases with which the grower has to contend. It is both a seed-bed and field disease, and as a storage trouble, it is especially destructive. Although control measures for the disease on the potato and in storage have been worked out rather satisfactorily, the grower of sweet-potato plants for sale purposes still has much trouble with it. In spite of all possible precautions he is likely to have a small amount of it develop in his plant beds. A single infected plant in a bundle of otherwise perfectly clean and healthy plants may, during shipment to the purchaser, serve to contaminate and infect the entire bundle in which the diseased plant occurs, or even the entire shipment. With the system of terminal inspection of parcel post and other shipments now in vogue in many States, such spread of infection in transit results in the condemnation and destruction or the rejection and return to the grower of many large lots of plants that, when packed and shipped by the grower, probably were apparently clean or at that time contained only a trace of infection. If such infection, limited at shipment to a single plant or so, could be held at that point without further spread during transit, the loss of plants by condemnation of shipments and the damage suffered by the purchaser when such lots escape such condemnation, due to escape from or lack of requirement of such terminal infection, would be reduced many hundredfold.

The grower who receives infected plants suffers threefold injury therefrom: (1), the death of infected plants that die without becoming established; (2), the infected plants if they live, will produce black-rotted potatoes; and (3), whether they live or not, will give rise to wholesale soil

infestation that will render the soil unfit for future crops of sweet potatoes. This third consequence is by far the most serious, since the pathogen will persist in the infested soil for an indeterminate number of years even in the absence of sweet-potato crops.

For years growers have been making enquiries indicating a desire for some form of plant treatment that might eliminate or at least check the dissemination of the black-rot organism while the plants are in transit from grower to purchaser. In view of these desires of the growers and in the light of reports of work done in recent years at the North Carolina Agricultural Experiment Station by Poole and Woodside,<sup>1</sup> who have shown that by dipping the stems and roots of sweet-potato plants in concentrated chemicals, infection by the sweet-potato wilt or stem-rot organism, *Fusarium batatas* Wr., might be prevented without killing the plant, it was decided to make some preliminary tests. Although a diverse assortment of materials and several strengths of Bordeaux mixture were tested by the North Carolina workers, their final conclusion was that a 20-20-50 Bordeaux mixture and a 25 per cent monohydrated copper sulphate-lime dust gave best results, with least injury to the plants treated. In their work, however, the plants were treated immediately prior to being transplanted to the field.

#### EXPERIMENTS IN 1932

In the spring of 1932 a few tests were designed to determine the degree to which sweet-potato plants would withstand the treatments mentioned in the foregoing when applied by the grower prior to their shipment to the purchaser. Plants to be used were purchased from a Mississippi grower and were of A-Grade quality, according to the certification system of the State Plant Board of Mississippi. The A-Grade certificate indicated that no black rot or stem rot whatsoever had ever been found on the premises of the grower, either in the field, storage, or plant-bed inspections as made by the regular State Plant Board inspectors.

One hundred plants of the Nancy Hall variety were dipped, stem and roots, in a 20-20-50 Bordeaux mixture solution and then allowed to drain. They were then tied up in bundles of 25 plants each. These 4 bundles were then wrapped together in a piece of newspaper, the tops alone remaining exposed. A similar number of plants were dipped into a copper-lime dust containing 25 per cent monohydrated copper sulphate. It was found unnecessary to moisten the plants, since the dust adhered to the stems and roots in surprisingly large amounts. These, likewise, were tied up in bundles of 25 plants each and wrapped in newspaper, as were the others. An additional 100 plants were divided into bundles and wrapped in a similar man-

<sup>1</sup> Poole, R. F., and J. W. Woodside, A chemical control for sweet potato wilt or stem rot. North Carolina Tech. Bul. 35. 1929.

ner, but with no treatment, to serve as checks. These 300 plants, representing 100 for each of the 2 treatments and 100 for the check, were then packed in a perforated box, such as is customarily used for shipping sweet-potato plants, and the box was set aside on a bench in the laboratory at ordinary room temperature for 5 days. A similar lot of 300 plants were treated and packed in an identical manner 2 days later and allowed to remain in the laboratory for 3 days. These periods of 3 and 5 days were supposed to represent periods of time during which the plants would be in transit from the grower to the purchaser. The 5-day period was chosen as a probable maximum during which plants would ordinarily remain in transit and storage between the time of leaving the grower and the time of being planted out in the field by the purchaser, such as might occur when shipped or received on a week end. The 3-day period was selected as a probable average time for such transit and storage between being pulled by the grower and transplanted to the field by the purchaser.

On June 20, 1932, both lots were planted in the field. The soil of the plot was a rather stiff, dark clay loam, with a small percentage of sand. It was realized that this was not the best type of soil for sweet potatoes, but it was the only land then available. It was rather dry, so that it was necessary to water the plants as they were transplanted. When unpacked the plants that had been held 5 days, packed for shipment, were somewhat yellowish, and the check plants, those not dipped in either the copper-lime dust or the Bordeaux mixture, were somewhat flaccid. The plants whose roots and stems had been dipped in either of these materials seemed considerably more turgid and in better condition generally than did the check plants, stored equally long, but without preliminary treatment. All the plants packed and stored for the 3-day period were green and turgid and in excellent condition for transplanting.

TABLE 1.—*Loss of stand in 1932 as a result of treatment prior to packing for shipment*

Stand on August 2, 45 days after planting

Treatment	After 3 days in storage	After 5 days in storage	Planted immediately no storage
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
20-20-50 Bordeaux mixture . . .	100	98	100
25% Copper-lime dust . . . . .	98	100	100
Check, non-treated . . . . .	98	97	100

Although no rain fell until July 2, 13 days after planting, no additional water, except that given at planting time, was ever applied to these plants. Some of the plants in each treatment, including the checks, appeared to be suffering severely. The larger percentage, however, seemed to stand up surprisingly well under the treatment to which they had been subjected. After the rain of July 2 most of the plants that had previously seemed to be dying began to revive and to put out new growth.

It will be noted from the above table that the loss of stand was negligible, even under the extremely severe treatment to which the plants had been subjected. The highest losses, in fact, were in the nontreated plants, excepting those held for 5 days in storage before planting. The next highest losses fell equally on the lots treated with Bordeaux mixture and stored for 5 days, on those treated with copper-lime dust and stored for 3 days, and on the nontreated lot stored for 3 days.

No differences in amount of vine growth throughout the growing season were apparent between the different treatments. Vine growth was exceptionally heavy and remained green and vigorous until late fall. At digging time, however, the yield proved very scanty, irrespective of the treatment accorded the plants. In consequence, no harvest records were taken. The potatoes had, in the parlance of the grower, all "gone to vine." This could not be attributed to any treatment at planting time, since it was common to all, even to those that had received no treatment whatsoever. It is more probable that the abundant vine growth and lack of potatoes were due to the unsuitable nature of the soil on which they were planted and probably in some measure to weather conditions prevalent during the growing season, since the fall season was extremely wet.

The results of this test were interpreted as being extremely favorable, indicating that sweet-potato plants possessed a marked degree of resistance to injury from treatment with highly concentrated copper compounds.

Another test was conducted simultaneously with the one above reported with the object of determining the efficacy of the same copper compounds in the prevention of infection by the black-rot organism. A culture of the black-rot organism, *Ceratostomella fimbriata*, was obtained from a specimen of sweet potato of the variety Porto Rico collected at Kosciusko, Attala County, Mississippi, in August, 1931, and maintained thereafter on a potato-dextrose-agar medium. New cultures were prepared on a cooked sweet-potato medium 4 days before use in inoculation tests. It was found that on the sweet-potato medium there was an abundance of microspores produced for use in making spore suspensions in a very short period of time.

On June 20, 1932, a lot of 100 plants of the Porto Rico variety was inoculated by dipping the roots and stems in a heavy suspension of spores of *Ceratostomella fimbriata* in distilled water. Another 100 plants were used

as checks, being merely dipped into clear distilled water before being planted. Still another 100 plants were first dipped into the spore suspension and, after being allowed to drain off thoroughly, were subsequently dipped as to stems and roots in a 20-20-50 Bordeaux solution before planting. A fourth lot was dipped in the spore suspension and, after drying, immersed into a 25 per cent copper-lime dust.

Thirteen days later, July 12, 100 per cent of the plants inoculated with black rot, but not treated with either of the copper compounds, showed black-rot infection. None of the check plants, dipped in distilled water, nor of the plants inoculated and subsequently treated with either the Bordeaux mixture or the copper-lime dust, ever exhibited any symptom of infection at any time. This test indicated that both treatments were very effective in preventing infection of the plants by surface-borne spores of the black-rot organism. These results, taken in conjunction with those obtained in the previously recorded test, presented such favorable promise of an effective method of prevention of the spread of black rot during transit of plants from grower to purchaser that it was decided to repeat the tests in the spring of 1933 to see whether or not they could be confirmed.

#### EXPERIMENTS IN 1933

The same strain of the black-rot organism as that employed in 1932 was used in 1933. New cultures were prepared on a cooked sweet-potato medium a week in advance of inoculation tests, and an abundance of spores were available at that time for making spore suspensions. The soil selected for the test, containing a considerably higher proportion of sand, was much better adapted to the growth of sweet potatoes than was that of 1932. The plants were grown in beds in the greenhouse from A-Grade seed of the Nancy Hall variety. A-Grade seed is apparently clean seed secured from a grower on whose premises no black rot or stem rot has been found, either in plant-bed, field or storage-house inspections, by the authorized inspectors of the Mississippi State Plant Board. The preparation of the soil and cultivation of the crop were similar to the practices commonly in use in commercial sweet-potato culture.

On May 10, 3 lots of 100 plants each were dipped in a heavy suspension of spores of the black-rot organism. After thorough draining, they were wrapped in newspaper, the tops only being left exposed, packed in a shipping box, and stored for 5 days in the laboratory. Three additional lots were dipped in sterile distilled water and wrapped and stored in a similar manner. Six other lots were dipped into the spore suspension. After having drained thoroughly, 3 of them were immersed in a 20-20-50 Bordeaux solution. After having dried, the other 3 were thoroughly dusted with a 25 per cent copper-lime dust. The plants given the Bordeaux treatment had their roots and stems completely immersed in the solution, only the tops re-

maintaining free from the chemical. Those given the dust treatment were covered tops and all by immersing them entirely in the dust. These lots were then wrapped and stored for five days. On May 12, 3 lots of 100 plants each were subjected to each of the respective treatments described above, the only difference being that the plants treated on this date were placed in storage for 3 days only.

On May 15, all of the plants that had been in storage for the 5- and 3-day periods were removed from storage and transplanted to the field. On many of the plants dipped in the spore suspension and stored for 5 days, visible evidence of infection was already in evidence. Such visible symptoms were not observable on those similarly treated but stored for 3 days only. A small percentage of the plants dipped in the spore suspension and stored for 5 days showed the typical black lesions of black rot, and under the hand lens the seta-like necks of perithecia of the causal organism were visible. None of those dipped in the spore suspension and subsequently treated with either the Bordeaux mixture or the copper-lime dust exhibited any sign of infection.

On May 15, 3 additional lots of 100 plants were subjected to one of each of the treatments described above, with the exception that the storage period was omitted. That is, 3 lots were dipped into the spore suspension, 3 were dipped into sterile distilled water, 3 into the spore suspension and then into the Bordeaux mixture, and 3 into the spore suspension and subsequently into the copper-lime dust. All were then planted simultaneously with those lots that had been stored 3 and 5 days, respectively.

The soil was in a finely pulverized condition and so dry at planting time that it was considered necessary to apply a small amount of water about each plant when transplanted to the field. A light rain fell some 10 days later.

As will be seen from table 2, a good stand was secured in all cases except

TABLE 2.—*Infection and stand count as a result of plant treatment, 1933*

Stand, July 1, 45 days after planting				Infection, July 1, 45 days after planting		
Treatment	Storage period			Storage period		
	none	3 days	5 days	none	3 days	5 days
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Check, distilled water .....	100	100	98	0	0	0
Spore suspension .....	16	17	2	89	100	100
Spore suspension and 20-20-50 Bordeaux mixture .....	100	99	98	0	1	0
Spore suspension and 25% copper-lime dust .....	99	100	97	1	0	1.5



where the plants were inoculated with the black-rot organism by dipping them in the spore suspension with no subsequent disinfection. In this case the stand was almost destroyed before the end of the 45-day period. At that time the few remaining plants in the plot were destroyed in order to limit as much as possible the spread of the black-rot organism to the disease-free plots. The table shows also that both the 20-20-50 Bordeaux mixture and the 25 per cent copper-lime dust gave almost complete control of black rot, even when the plants were thoroughly inoculated by dipping them in a heavy spore suspension.

Yield records at the end of the season, as shown in table 3, indicate that treatment of plants with either 20-20-50 Bordeaux mixture or the 25 per cent copper-lime dust did not injure the plants beyond their ability to recover under the conditions of the test. In fact, the average yield of the rows treated with these materials and planted immediately was higher in each case, though not beyond the limits of experimental error, than that of the plants merely dipped in distilled water before planting. The yield from the plants treated with 25 per cent copper-lime dust and stored for 3 days was essentially the same as that from plants dipped in water and stored for the same length of time, while those treated with the 20-20-50 Bordeaux mixture and stored for the same period yielded only slightly less. The yield in each case where the plants were stored for 5 days was lower than that from the corresponding treatment, when the plants were planted immediately without undergoing a period of time in shipment or storage. The reduction, however, was nearly as large in the case of the plants dipped in water only prior to storage as in that of plants treated with either Bordeaux mixture or copper-lime dust and stored. It is somewhat of a surprise to note that the yield from plants stored for 5 days was slightly higher than when similarly treated plants were stored for 3 days only. These differences are all very small, however, and are all within the limits of experimental error, hence have no significance.

#### DISCUSSION

These results secured from 2 consecutive years of experimentation under field conditions seem to indicate that treatment of sweet-potato plants by the grower, at time of pulling, with either a 20-20-50 Bordeaux mixture or a 25 per cent copper-lime dust may be practiced without material detriment to the plants. The time period of 5 days is ordinarily as long as would occur between treatment by the grower and transplanting by the purchaser. The fact, however, that the yield from plants stored for 5 days was as large as or even larger than from plants stored for 3 days might indicate that the time necessary to shipment and storage might possibly be safely extended even beyond the 5-day period employed in these tests.

TABLE 3.—*Sweet-potato-plant treatment, yield in pounds per 100 plant row, 1933*

Treatment	No storage				Stored 3 days						Stored 5 days						
	Ser. 1		Ser. 2		Ser. 3		Ave.		Ser. 1		Ser. 2		Ser. 3		Ave.		Ave. yield
	Ser. 1	Ser. 2	Ser. 1	Ser. 2	Ser. 1	Ser. 2	Ser. 3	Ave.	Ser. 1	Ser. 2	Ser. 1	Ser. 2	Ser. 3	Ave.			
Check, distilled water . . . . .	138.5	142.0	128.0		136.2	118.0	156.0	126.0	133.3	155.5	123.0	118.0	132.2			133.9	
Spore suspension and 20-20-50 Bordeaux mixture	114.5	154.0	148.0		138.8	151.0	124.0	116.0	130.3	125.0	130.5	138.5	131.0			133.4	
Spore suspension and 25 per cent copper-lime dust	127.5	168.0	126.0		140.5	137.0	118.0	146.0	133.7	104.5	137.5	151.0	131.0			135.1	

The weather conditions in both years of these tests were more unfavorable to the growth of sweet-potato plants at transplanting time than are encountered in the average season, being unusually dry in both cases. Both stand percentages and yield records show that the plants treated with the chemicals are able to withstand these unfavorable conditions almost as well as nontreated plants that have been stored equally long.

These tests also show that either of the above treatments is very efficient in the prevention of infection of the plants by surface-borne spores of the black-rot organism, even under the extremely favorable conditions for infection induced by dipping the plants in a heavy spore suspension of black-rot spores and then subjecting them to long confinement in the close atmosphere of the shipping container. It would seem, therefore, that this is a method whereby spread of black rot on sweet-potato plants may be prevented during the time of shipment from the grower to the purchaser, the interval during which most of such spread normally occurs. It would seem that this method is an adequate answer to the insistent demand on the part of the grower that has been heard so constantly and repeatedly since the advent of sweet-potato-plant inspection, a demand calling for some method whereby this spread of infection may be avoided.

It will be necessary, of course, to educate the purchaser to the acceptance of plants coated with a heavy covering of liquid or dust, which, especially in the case of the Bordeaux mixture, renders them somewhat unsightly. If, however, he can be adequately convinced that such plants will live as well on being transplanted in the field, will produce as well as plants not so treated, and will also yield him a disease-free crop and avoid the introduction into his soil of the persistent and destructive black-rot organism, he will soon become an enthusiastic advocate of such treatments, regardless of the appearance of the plants. If the method works out as well in actual commercial practice as it has in these 2 years of field experimentation, it should prove of great value to the plant producer by eliminating the loss of many shipments that are normally returned to him or destroyed by the plant-inspection agencies. It should result in increased good-will toward him on the part of his customers, since it would eliminate many complaints from disgruntled purchasers that had paid for clean plants and had instead harvested a heavily infected crop of potatoes and, worse still, had destroyed for several years the value of their fields for subsequent sweet-potato production.

The effect of such treatments, when applied to clean plants planted on infested soil, has not yet been determined. Poole has found that such treatments retard, or at least delay very materially, the infection of sweet-potato plants by the stem-rot or wilt organism, *Fusarium batatatis* Wr. By analogy, therefore, it is quite probable that plants so treated would be protected,

at least for a time, and that infection would be delayed in infested soils so that a smaller amount of black-rotted potatoes would result in the harvested crop.

#### SUMMARY

Tests were made over a period of 2 consecutive years to determine the degree of resistance of sweet-potato plants to treatment with a 20-20-50 Bordeaux mixture solution and with a 25 per cent copper-lime dust. Entire plants immersed in the copper-lime dust and plants with their stems and roots dipped in the concentrated Bordeaux mixture were but slightly affected, either as to stand or yield, when planted immediately after treatment.

Plants inoculated by dipping their stems and roots into a heavy suspension of spores of the sweet-potato black-rot organism, *Ceratostomella fimbriata*, showed a very high percentage of infection when planted immediately. One hundred per cent infection resulted when plants so inoculated were wrapped in bundles and stored for 3- and 5-day periods in a container under conditions such as they would normally undergo during transit from grower to purchaser.

When plants were inoculated by dipping their stems and roots in a black-rot spore suspension and were subsequently treated with either the Bordeaux mixture or the copper-lime dust, infection was almost entirely eliminated, even when the plants were stored in the close atmosphere of the shipping container for periods of 3 and 5 days.

Yields from sweet-potato plants treated with the above materials and stored for 3 and 5 days in a shipping container were but slightly reduced over those plants similarly treated but with the storage period omitted. The yields from plants treated with the chemicals and not stored before planting were slightly higher than from plants merely dipped in water and planted. Plants treated with the disinfectant materials and stored for 3 and 5 days gave no significant difference in yield over plants dipped in water and stored for the same length of time.

These results have made it apparent that sweet-potato-plant growers can treat their plants by dipping them in a 25 per cent copper-lime dust or by immersing their stems and roots in a 20-20-50 Bordeaux solution prior to shipment to the purchaser without materially injuring them, provided the time required for shipment and storage be no longer than 5 days. These tests have not shown the limit of time that plants so treated will endure without injury in storage and transit. Treatment with either of the materials tested will practically eliminate the dissemination and spread of black rot on sweet-potato plants during transit from the grower to the purchaser.

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# SOME PRELIMINARY FEEDING EXPERIMENTS WITH SCABBY BARLEY<sup>1</sup>

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In 1928 barley in the Midwestern States suffered from a severe epidemic of scab caused by *Gibberella saubinetii* (Mont.) Sacc. The same year some of the barley raised in this region was exported to Belgium, Holland, and Germany where it was used for feeding hogs. Grain-exporting firms received complaints, especially from Germany, that the barley was causing disease symptoms in hogs and that such diseased hogs refused to continue eating rations containing this barley.

Scott (9) reported the presence of wheat scab in Iowa as early as 1866, indicating its presence in the State for at least 65 years, and records<sup>3</sup> for the period since 1908 show that a slight amount of scab has been present on barley, except in the years 1919, 1928, and 1932, in which there was a loss in crop yield of from 4 to 5 per cent. It was not until 1928, when there was a loss of 4 per cent, that difficulty was encountered in feeding the grain.

After harvesting the 1928 crop the Iowa Agricultural Experiment Station received numerous complaints regarding the ill effects of feeding scabby barley to hogs in Iowa. Some complaints also were received that chickens would not eat barley and that where chickens ranged in barley fields, either before or after harvest, they became ill.

Grain infected with microorganisms has been known to induce injurious effects to man and animals in Europe and to a lesser degree in this country; but, in the United States, the seriousness of the problem in 1928 exceeded all previous records. This condition of our barley grain led to an investigation, commencing in the fall of 1928, of the effect of scabby barley as a feed for hogs, chickens, and guinea pigs. At the 1929 meeting of the American Phytopathological Society the following results were reported in an abstract by Mundkur and Cochran (5). "Hogs fed on an exclusive diet of this barley developed nausea for food and starved rather than eat it, while checks fed on clean barley were not sick and showed a slight increase in weight. After the fifth day the test animals fed on scabby barley began to vomit, but did not develop diarrhea.

"Mature chickens fed on the scabby barley showed no disease and lost no weight even when they were fed on artificially infected barley; whereas

<sup>1</sup> Journal paper No. J137 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 5. (Old series).

<sup>2</sup> The writer wishes to express his gratitude to Dr. I. E. Melhus for suggestions and assistance in the experimental work and in preparation of the manuscript.

<sup>3</sup> Plant Disease Survey.

chickens two weeks old showed a loss of weight and developed a rough plumage. The birds were not on an exclusive barley diet and it was observed that they rejected a lot of feed, picking out, as far as possible, the mash.

“Guinea pigs rejected an exclusive barley diet. Fed on a half-and-half mixture of scabby barley and mash, they lost in weight, but did not develop any disease symptoms such as was exhibited in the hogs.”

At the meeting referred to above, Roche, Bohstedt, and Dickson (8) reported as follows: “The farm utilization of scab-infected barley has been found economical by feeding it to cattle, sheep and poultry. The ruminants and poultry make good gains on heavily scabbed grain with no apparent ill effect. Pigs, horses, and dogs, as well as man, are very sensitive to the accumulated products in the infected grains and will not tolerate low percentages of badly scabbed kernels. No method has been found to date whereby badly scabbed grain can be fed economically to pigs.”

Miessner and Schoop (4) noted that when macerated fungus (*Gibberella saubinetii*) material, as well as the liquid in which the fungus had grown, was injected subcutaneously into hogs, it caused violent vomiting and acute paralysis. Mice and guinea pigs did not develop these symptoms, a fact that agrees with the results of feeding experiments with guinea pigs reported in this paper.

In 1930 Roche and Bohstedt (7) found that lambs and ewes showed no apparent difference, whether the grain upon which they were fed was diseased or normal. Scabbed oats carrying 70 per cent infection, if they exceeded 60 per cent of the grain ration, were found to be unpalatable to horses.

In 1930 Mains *et al.* (3) reported that scabby barley had been fed to chickens without causing decrease in weight, but that hogs lost if the amount of scabby barley exceeded 10 per cent. Barley containing 58 per cent scab seemed to have no injurious effect on cattle.

#### EXPERIMENTAL STUDIES

*The Causal Organism.*—The diseased grain does not have the attractive pale straw color of healthy bulk barley, but is slightly dark brown in appearance. The tip of the caryopsis is bluish black or very deep brown, which gives the slightly dark brown color to the grain in mass. The grains are shrivelled, and the bluish black tips, when examined with a hand lens, show small black granular bodies occurring in groups on the surface, the perithecia of a pyrenomycetous fungus. The perithecia are ovoid or subconical; each contains over one hundred asci, and each ascus contains eight spores. The fungus was identified as *Gibberella saubinetii* (Mont.) Sacc.

Hyaline hyphae were demonstrated in diseased grains whether they showed perithecia or only brownish tips.

*Tests with Hogs.*<sup>3</sup>—Six hogs were available for experimentation. The initial weight of the hogs was recorded and they were starved for 24 hours. After this compulsory fast they were given rations as follows: Four hogs were given naturally-infected barley showing about 40 per cent of the grains discolored by the scab fungus; 2 hogs serving as checks were given clean, healthy barley.

The infected barley was made into meal and mixed with warm water at the time of feeding. The hogs were fed twice daily. On the first day the feeds were consumed without any apparent hesitation. On the second day the early morning feed was consumed but thereafter it was refused. Substitution of milk for water induced the hogs to consume a part of the feed but they took it reluctantly. After the sixth day they absolutely refused the feed. The hogs fed on clean barley made no objection to their rations. The hogs on scabby barley would not drink even the milk that separated to the top, after the mixture had stood for a while. Vomiting commenced after the sixth day and continued 10 days, when the experiment was terminated, as it was considered inadvisable to starve the animals further. The weights of the animals before and after the experiment are given in table 1.

The losses in weight recorded in table 1 probably should be considered largely due to the starvation of the hogs. The barley induced vomiting but did not cause diarrhea or enteritis. Further results were irritation of

TABLE 1.—*The effect of feeding scabby barley for 10 days on the weights of two lots of hogs*

Lot	Animals	Weight			
		Grain	Initial	Final	Gain or loss
No. 1	No. 1	Scabby barley	Lbs. 102	Lbs. 88	Lbs. - 14
	" 2	" "	60	52	- 8
	" 3	" "	88	71	- 17
	" 4	" "	70	58	- 12
No. 2	" 5	Clean barley	68	68	0
	" 6	" "	83	88	+ 5

<sup>3</sup> The author is indebted to Professor Charles Murray, Department of Veterinary Pathology, for assistance in carrying out these tests, which were conducted by the Veterinary Division of the Iowa State College.

the alimentary canal and some kind of intoxication. Sloughing of hoofs or shedding of hair did not take place. The 2 animals held as checks and fed on clean barley appeared healthy and did not lose weight. One of them gained 5 pounds. These results are in line with those reported from Germany by Oppermann and Doenecke (6) and Beller and Wedemann (1) who noted nausea and vomiting, losses in body weight, and some degree of intoxication. Beller and Wedemann considered that the disease-causing agent is to be sought for in the endosperm and the glumes and that it is possibly connected with a volatile substance that hogs can detect.

*Experiments with Chickens.*<sup>5</sup>—While the hog experiments were in progress, three similar feeding trials with chickens were undertaken. The chicks were 2, 6, and 8 weeks old, respectively. No attempt was made to provide a balanced ration. To the standard mash different proportions of barley, naturally infected and artificially infected,<sup>6</sup> were added as indicated in the table. The test lasted for fourteen days during which time the birds were under continuous observation. The results of the experiment are recorded in table 2.

Observations made during the experiments indicate that scabby barley, although not palatable to growing chicks, is not poisonous to them: (1) that, while chicks fed on standard mash alone gained in weight, those fed on rations containing scabby barley failed to gain, or made smaller increases in weight, due in part to voluntary starvation; (2) that artificially infected barley was more injurious than naturally infected barley, and (3) that the

<sup>5</sup> These tests were conducted in cooperation with the Poultry Husbandry Department through the assistance of Professor R. L. Cochran.

<sup>6</sup> Preparation of single-spore cultures: Perithecia were collected from infected kernels and crushed in a clean agate mortar. The ground material was suspended in water. By the dilution-plate method of Keitt (2) monosporic ascospore isolations were made. After 12 to 14 hours at 26° C. the ascospores had germinated and the germ tubes had grown sufficiently long to make possible the selection of single spores which were isolated to potato-agar slants, furnishing monosporic cultures for experimental use.

Preparation of meal cultures: For the feeding tests with the fungus, cultures were made on barley from the previous year's crop (1927), in which the infestation was very slight. The grain looked healthy and only rarely could a grain be found that had the bluish-black perithecia-covered tips.

To prepare the cultures, the barley was first soaked in water for 24 hours; then it was placed in Mason jars. Each jar was filled about three-fourths full, plugged with cotton, covered with Kraft paper caps, and autoclaved for 3 hours at 15 pounds' pressure. Three days afterwards when it was found that sterilization was complete, the contents of the jars were inoculated with the fungus from the agar slants.

The fungus was allowed to grow for 35 days, by which time the barley was completely infected. The jars were then emptied and the material was quickly air-dried by use of an electric fan, after which it was made into a meal in an electric grinder. The meal, which had a wine red color, was stored in clean dry Mason jars in the ice box until it should be used.



TABLE 2.—*Effect of a scabby-barley diet for 14 days upon the weight of chicks*

Experiment	Lot no.	Number of chicks	Ration	Weight in pounds		
				Initial	Final	Gain or loss
I. Chicks 2 weeks old	9	6	Half culture scabby wheat; half standard mash .....	0.98	1.08	+ 0.10
	10	6	Half culture scabby barley; half standard mash .....	0.98	1.18	+ 0.20
	11	6	Naturally scabby barley .....	0.98	1.56	+ 0.58
	12	6	Culture scabby barley .....	0.98	1.10	+ 0.12
	13	6	Check: standard mash .....	0.98	1.52	+ 0.54
II. Chicks 6 weeks old	1	20	Half naturally scabby barley; half standard mash ..	9.76	13.64	+ 3.88
	2	20	Three-fourths naturally scabby barley; one-fourth standard mash .....	9.60	11.24	+ 1.64
	3	20	Check: standard mash .....	9.80	16.34	+ 6.54
III. Chicks 8 weeks old	4	6	Half culture scabby wheat; half standard mash .....	6.26	6.90	+ 0.64
	5	6	Half culture scabby barley; half standard mash .....	6.00	6.74	+ 0.74
	6	6	Naturally scabby barley .....	5.94	7.22	+ 1.28
	7	6	Three-fourths culture scabby barley; one-fourth standard mash .....	5.30	5.10	- 0.20
	8	6	Check: standard mash .....	6.20	7.84	+ 1.64

lots receiving the highest percentage of artificially scabby barley seemed to be injured most, as indicated by the loss in weight of the birds and also by the rough and ragged conditions of their plumage.

*Experiments with Guinea Pigs.*—Experiments with guinea pigs were begun in the winter of 1929. It was thought that these animals would respond quickly to the different diets, but the results belied the expectations.

Eleven animals were placed for a period of 15 days on a standard diet, consisting of finely ground yellow corn, alfalfa meal, flour middlings, powdered buttermilk, bone meal, and a small quantity of common salt. After 14 days records were taken of their weights. For the test the feeding was apportioned as follows: Three guinea pigs—standard mash plus clean barley, mixed in equal portions; 2 guinea pigs—standard mash plus naturally infected barley, equal proportions; 4 guinea pigs—standard mash plus artificially infected<sup>7</sup> barley, equal proportions; 2 guinea pigs—standard mash alone (checks). The experiment continued for a period of 25 days. The results are recorded in table 3.

The animals that were fed on barley, either clean or scabby, did not

<sup>7</sup> Artificially infected barley was prepared as in case of the experiment with chickens.

TABLE 3.—*Effect of a diet consisting of standard mash and scabby barley for 25 days on the weight of guinea pigs*

Lot	Number of animals	Ration	Weight in grams		Gain or loss in per cent
			Initial	Final	
1	3	Half mash, half clean barley	3177	3098	- 2.49
2	2	Half mash, half scabby barley .....	2407	2034	- 15.94
3	4	Half mash, and half artificially infected barley.....	4329	3807	- 12.06
4	2	Standard mash (check) .....	2030	2094	+ 3.15

show any signs of disease during the course of this experiment, but were lively and frisked about like those of the check lots. However, all the animals that received any barley in their rations lost weight. Barley is not relished by guinea pigs and, at least in such high proportions, is not a good diet for them. Animals receiving scabby barley, either naturally or artificially infected, lost more heavily than those receiving clean barley. It was observed that they ate the rations rather reluctantly and much food was left over after each feeding. Possibly the loss in weight was due to this voluntary starvation. Scabby barley apparently did not induce disease even though the animals were forcibly fed.

After this test, the animals were restored to a normal ration consisting of the standard growing mash. They were weighed at the end of 20 days and these weights were recorded as the initial weights for a second test in which the quantity of barley was raised to 75 per cent. This ration, however, was entirely refused by the animals and, after 3 days, to save the animals unnecessary starvation, the experiment was terminated.

#### SUMMARY

The 1928 crop of barley in the Midwestern States was injured by a severe epidemic of scab due to *Gibberella saubinetii*, and reports that scabby barley caused disease in hogs and chickens were received by the Iowa Agricultural Experiment Station.

Feeding tests with hogs showed that 40 per cent scabby barley was distasteful, and caused vomiting and slight intoxication. There were no other serious complications, such as diarrhea or enteritis.

Poultry fed on scabby barley did not show disease symptoms, although feeds containing barley were not relished.

Guinea pigs lost in weight when put on a half-and-half barley-stand-

ard-mash diet, and while they lost more when the barley used was scabby, they did not become ill. Clean and scabby barley seemed to be equally distasteful to the guinea pigs.

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# ENZYMES OF THE RHIZOMORPHS OF *ARMILLARIA MELLEA*

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(Accepted for publication January 2, 1934)

Although considerable work has been done on the presence of enzymes in the mycelium and sporophore of *Armillaria mellea* (Vahl.) Sacc., the writer has found no record of any having been done on the enzymes of the rhizomorphs. Kohnstamm (3), working with the mycelium and sporophores, reported the presence of diastatic, proteolytic, glucoside-splitting, and cellulose-hydrolyzing enzymes. Schmitz and Zeller (5), in their study of the mycelium, reported the presence of maltase, lactase, sucrase, raffinase, diastase, inulase, cellulase, hemicellulase, emulsin, urease, amidase, trypsin, and erepsin. Gerber (1), demonstrated the presence of rennet in 86 different species of fungi, including *A. mellea*.

## MATERIALS AND METHODS

Material was secured from a shallow well that was curbed with Douglas fir lumber. The rhizomorphs grew out into the water from the curbing until a mat was formed over the surface of the water and to a depth of several feet. These rhizomorphs could be gathered by reaching down from the top of the well; thus, material that was free of foreign matter was obtainable in large quantities. It is not known whether the source of food was the curbing of the well, or other organic material near at hand. Hotson (2) has reported the growth of the rhizomorphs in mines and wells, stating that the growth is very abundant.

Two methods were employed in preparing the rhizomorphs for extraction of the enzymes. The first consisted of drying the material at room temperature by means of an electric fan. The dry rhizomorphs were then ground several times in a meat grinder. A known weight of the ground material was then placed in a flask and 4 times that weight of distilled water was added, with a small amount of chloroform as an antiseptic. The mixture was allowed to stand over night to extract the enzymes. It was then filtered through a Buchner funnel, and the enzymes were precipitated from the filtrate with 3 volumes of 95 per cent alcohol. The precipitated enzymes were then collected on filter papers in a Buchner funnel, and the papers allowed to dry at room temperature. The papers were kept in glass-stoppered bottles in the dark until needed.

The rhizomorphs were so very tough and stringy that considerable material was lost in grinding. It also was found that the material was not so

<sup>1</sup> The writer is indebted to Dr. J. W. Hotson and Dr. G. B. Rigg for advice and helpful criticism in conducting the work reported in this paper.

finely ground as was desired. In order to overcome these difficulties, a second method was employed, essentially the same as the first, except that the rhizomorphs were ground in a mortar while they were still fresh. Before grinding, a little sand and distilled water were added to facilitate the process. After the mass had been ground to a fine pulp, enough distilled water was added to bring the water content up to approximately 4 times the weight of the rhizomorphs, had they been dried. The remainder of the process is identical with that described above.

When the enzyme material was to be used, the filter papers were soaked in a quantity of water so that 1 cc. of the resulting enzyme solution would be equal to one gram of the fresh rhizomorphs. For instance, 10 grams of material fresh from the well would, when precipitated on the filter papers, require 10 cc. of distilled water at this stage of the procedure.

All experiments, unless otherwise described, were set up in the following manner: 10 cc. of the prepared substrate was put into each of 3 test tubes. Two cc. of the enzyme solution was added to the first tube; to the second, was added 2 cc. of the enzyme solution that had been autoclaved at 10 pounds' pressure for 10 minutes; to the third tube was added 2 cc. of distilled water.

If the results of the experiment were positive, the first tube would show positive results, while the second and third tubes, the controls, would show negative results. Toluol was used as the preservative in all experiments that were allowed to run for more than a few hours.

TABLE 1.—*Results of experiments conducted to detect the presence of enzymes in the rhizomorphs of Armillaria mellea*

Test for	Substrate	Incubation period	Result
Diastase	Potato starch	4 hours	+
Inulase	Inulin	3 days	+
Hemicellulase	Date endosperm		
	Van Tieghem cells	45 "	-
	Test-tube method	30 "	-
Invertase	Sucrose	1 day	+
Maltase	Maltose	25 days	-
Lactase	Lactose	25 "	-
Rennet	Milk	36 hours	+
Oxidase	Guaiacum	2 "	+
Peroxidase	"	2 "	+
Catalase	Hydrogen peroxide		+
Esterases	Olive-oil emulsion	25 days	-
"	Ethyl acetate	25 "	-

TESTS OF THE RHIZOMORPHS OF *ARMILLARIA MELLEA* FOR ENZYMES

**Diastase.**—Starch paste was used as the substrate. Potato starch was converted into a 0.25 per cent paste according to the following method: Two and one-half grams of potato starch in 150 grams of distilled water were brought to a boil while constantly stirred. It was then transferred to a flask containing about 600 cc. of hot distilled water. The whole was boiled in a reflux condenser for 2 hours. After cooling, the volume of the paste was made up to a liter by adding distilled water. One per cent toluol was added and the flask plugged until ready for use.

The experiment was set up as described above. The Fehling's test was applied after 4 hours.

**Inulase.**—A 1 per cent solution of inulin was used as the substrate. The experiment was set up as described in the foregoing. The tubes were incubated at 30° C. After 3 days the Fehling's test was applied.

**Hemicellulase.**—For this experiment, the endosperm of the date seed was used as the source of the hemicellulose. Date seeds were scraped to remove the outer coat. They were then scrubbed with sand and soap in order to remove all the reducing sugars possible, washed in distilled water, cracked, and the embryos removed. The endosperms were then autoclaved in distilled water at 15 pounds' pressure for  $\frac{1}{2}$  hour in order to destroy any enzymes that might be present. They were then rinsed and stored in distilled water until needed, with toluol as the preservative.

Two methods of testing for hemicellulase were employed. Very thin slices of the date endosperm were cut on the microtome and suspended in hanging drops in properly prepared van Tieghem cells. Cells were set up as follows: (1) Three cells with date endosperm suspended in a drop of enzyme solution; (2) three cells with date endosperm suspended in a drop of enzyme solution that had been autoclaved; and (3) three cells with slices of the date endosperm suspended in distilled water. Enough of the solution was placed in the bottom of each cell to prevent evaporation. Chloroform was used as an antiseptic. The cells were incubated at 30° C. and were observed from time to time for signs of erosion of the endosperm. Although the cells were observed over a period of 45 days, no erosion was detected.

The second method consisted of placing pieces of the date endosperm in each of 3 test tubes. To the first was added 10 cc. of the enzyme solution; to the second was added 10 cc. of the enzyme solution that had been autoclaved; and to the third was added 10 cc. of distilled water. Toluol was used as the antiseptic. The tubes were incubated for 30 days at 30° C., they were then tested for reducing sugars with Fehling's solution. Results were negative.

*Invertase*.—A 1 per cent solution of sucrose was used as the substrate. Tubes were set up as described above. They were allowed to incubate at 30° C. for 24 hours, then tested with Fehling's solution.

*Maltase*.—A 1 per cent solution of maltose was used as the substrate. Tubes were set up as described above and allowed to incubate at 30° C. for 25 days. They were then removed from the incubator and the Fehling's test applied. Since maltose reduces Fehling's solution, it was necessary to apply some quantitative test in order to determine the presence of the enzyme. One molecule of maltose is hydrolyzed into 2 molecules of glucose. From this fact it is possible to determine the presence of the enzyme by an increase in the amount of Fehling's solution reduced. The following method was used in determining the amount of Fehling's solution reduced: After applying the Fehling's test, filter through a Gooch filter, dissolve the copper oxide with 5 cc. of 6N nitric acid, wash with 10 cc. of distilled water, boil to expel red fumes, dilute to 25 cc., add 3N NaOH to permanent turbidity, clear the solution with 6N acetic acid, plus 1 cc. excess, dilute to 40 cc. with distilled water. Add potassium iodide crystals, titrate with sodium thiosulphate, using fresh starch solution as the indicator.

*Lactase*.—A 1 per cent solution of lactose was used as the substrate. The procedure was the same as for maltase.

*Rennet*.—Fresh cow's milk was used as the substrate. The experiment was set up as described above. The tubes were incubated at 30° C. for 24 hours, whereupon the first tube showed distinct curds, while the controls showed none. After 36 hours, the first tube was completely coagulated, while the controls showed no coagulation.

*Oxidase*.—In testing for oxidase, a 10 per cent solution of guaiacum gum was used. Since the gum oxidizes in the air, large pieces were selected. The outside of the pieces was cut off with a sharp knife so that only the interior of the lumps was used. The gum was dissolved in alcohol. Five cc. of the enzyme solution was placed in a test tube; in a second tube, 5 cc. of the boiled enzyme solution was placed. A few drops of the guaiacum solution was added to each tube. After two hours, the first tube showed the blue color, indicating the presence of an oxidase. The control showed no color for twelve hours.

*Peroxidase*.—The experiment was set up exactly as was the oxidase experiment. On adding the guaiacum-gum solution, no color developed, but on the addition of a few drops of ordinary hydrogen peroxide, a blue tint began to appear. In 15 minutes, a deep blue had developed in the first tube. The control showed no color even after standing for several hours.

*Catalase*.—When a few drops of hydrogen peroxide were added to 2 cc. of the enzyme solution, bubbles were formed in great abundance. When

this was repeated with the boiled enzyme solution, no oxygen was given off, thus indicating the presence of catalase.

*Esterases.*—Experiments were conducted using olive-oil emulsion and ethyl acetate as substrates. Olive-oil emulsion was made according to the method of Bloor. Ten cc. of olive oil was dissolved in hot absolute alcohol. This solution was run through a hot funnel, to which was attached a piece of glass tubing drawn out to a fine jet, into 100 cc. of constantly stirred, cold, distilled water. The milk-white emulsion was boiled to drive off the alcohol, and was diluted to 500 cc. with distilled water.

For the ethyl acetate experiment, a 1 per cent solution of ethyl acetate was used as the substrate.

Experiments were set up as described in the first part of this paper. The tubes were incubated for 25 days at 30° C. The contents of the tubes were then titrated with N/20 NaOH, using phenolphthalein as the indicator.

#### DISCUSSION

The list of enzymes for which tests were made is far from complete. The fact that negative results were obtained in some cases does not prove that the enzyme does not occur in the rhizomorphs. When positive tests were secured, they were repeated until there could be no doubt of the presence of the enzyme. It is not possible from the results so far obtained to make any positive statement as to the rôle played by the enzymes found in the rhizomorphs. Whether they are concerned with the entry of the rhizomorphs into the host was not determined, though it seems that such may be possible. Thomas (6), however, is of the opinion that the entry is largely due to mechanical pressure.

#### SUMMARY

The enzymes found in the rhizomorphs of *Armillaria mellea* are diastase, inulase, invertase, rennet, oxidase, peroxidase, and catalase; the enzymes for which tests were made and not found are hemicellulase, maltase, lactase, and the esterases.

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# INOCULATION TESTS WITH VERTICILLIUM WILT OF MUSKMELONS<sup>1</sup>

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(Accepted for publication January 8, 1934)

In late July, 1932, a serious wilt occurred in a planting of the Persian variety of muskmelons, *Cucumis melo* L., in San Joaquin County, California. The first evidence of the disease was the wilting of the crown leaves just before harvest; followed very soon by the wilting and death of the entire plant. Additional diseased plants continued to appear over a period of several weeks, with the resultant death of 15 to 20 per cent of the stand. These plants showed a brown discoloration in the vascular tissues of the root, stem, and large lateral shoots, and freehand sections showed the presence of mycelium in the discolored regions. Repeated isolations from the roots and stems of diseased plants yielded mostly pure cultures of a species of *Verticillium*.

The Persian melons were growing in the middle section of the field and on one side of this section, the Honey Dew variety of muskmelons, *C. melo* L. var. *inodorus* Naud., was growing, while the variety, Hale's Best, *C. melo* L. var. *reticulatus* Naud., was growing on the other side. No evidence of the disease showed in either the Honey Dew or Hale's Best plantings. The marked susceptibility of the Persian variety has been reported by Rudolph<sup>3</sup>.

The pathogenicity of the *Verticillium* isolated, identified by B. A. Rudolph as *V. albo-atrum*, was established in the fall of 1932 by growing Persian melons in 6-inch pots of steam-sterilized soil to which a heavy water suspension of *Verticillium* spores was added so as to wet the top to a depth of 2 inches. Additional successful inoculations were secured by placing fragments of agar from a culture of *Verticillium* in contact with wounded roots. The results, however, obtained by growing plants in infested soil, showed that wounding the roots was not necessary to produce the disease.

Under greenhouse conditions, the first symptoms of the disease were evident in approximately 6 weeks from date of planting and were manifest by a slight yellowing of the leaves near the crown and wilting and curling of irregular areas along the margin of the yellowed leaves. There was a somewhat slower progression of the wilt symptoms from the first foliage leaves to the growing tips of the vines than under field conditions, but eventually

<sup>1</sup> Contribution from the Division of Plant Pathology, Branch of the College of Agriculture, University of California, Davis, California.

<sup>2</sup> The writers are indebted to Professors Ralph E. Smith and Max W. Gardner for suggestions and criticisms.

<sup>3</sup> Rudolph, B. A. *Verticillium* hadromycosis. *Hilgardia* 5: 197-353. 1931.

the entire plant wilted and died. Diseased plants showed the characteristic vascular discoloration in the roots, stem, and branches, especially in the axillary leaf-petiole and branch tissue. In a critical study of 6 diseased plants, resulting from artificial inoculation, the causal fungus was recovered from all parts of the main shoots up to within a few inches of the growing tips.

Reference to the literature shows that Van der Lek<sup>4</sup> found a *Verticillium* associated with a melon disease in Europe as early as 1918. Gram and Rostrup,<sup>5</sup> in 1925, and Dufrénoy,<sup>6</sup> in 1927, also report the occurrence of a *Verticillium* disease of melons, but none of these workers gives any account of pathogenicity studies. Bewley<sup>7</sup> (pp. 83-84) isolated the fungus from melons and certain other hosts and obtained successful inoculations. In America, Rudolph (*loc. cit.*) isolated from diseased muskmelon plants a species of *Verticillium* that he considered to be *V. albo-atrum*.

The occurrence of the disease in a severe form on Persian melons in 1932 in a field where Hale's Best and Honey Dew varieties were growing without showing evidence of the disease, would lead one to suspect that these 2 varieties were possibly resistant to the fungus. Controlled greenhouse trials were carried out to determine the relative susceptibility of the common commercial varieties of muskmelons grown in California. Cultures of *Verticillium* growing on steamed oats were uniformly mixed into a large quantity of steam-sterilized soil with which 6-inch pots were filled. On February 21, 1933, 4 series of 16 pots each were planted with seed of 4 of the principal varieties grown in California, Hale's Best, Honey Ball (*Cucumis melo* L. var. *inodorus* Naud.) Honey Dew, and Persian. An additional series was started on March 7, consisting of 35 pots of the Casaba variety *C. melo* L. var. *inodorus* Naud., 16 pots of Hale's Best, 18 pots of Honey Ball, 20 pots of Honey Dew, and 18 pots of Persian. When the plants were putting out their first foliage leaves they were thinned to 4 plants per pot. In 6 weeks from date of planting, the first symptoms of wilting were evident and were comparable in every way with those secured in the earlier pathogenicity studies. For the records, which were taken on May 12 and June 1, each plant was examined carefully for wilt symptoms by cutting the stems and culturing from diseased plants. The results of the above tests are presented in table 1.

<sup>4</sup> Van der Lek, H. A. A. Ouderzoekingen over tracheomycosen: De verticilliose van den komkommer. Med. Landbouwhoogesch. Wageningen 15: 1-45. 1918.

<sup>5</sup> Gram, E., and S. Rostrup. Oversigt over sygdomme hos landbrugets og havebrugets kulturplanter, 1924. Tidsskr. Planteavl 31: 353-417. 1925.

<sup>6</sup> Dufrénoy, J. Déperissement des arbres fruitiers dans le Massif Central. Bul. Off. Agr. Rég. Massif Central Clermont-Ferrand 7: 103-113. 1927.

<sup>7</sup> Bewley, W. F. Diseases of glasshouse plants. 208 pp. E. Benn, limited, London. 1923.

TABLE 1.—*Susceptibility tests of 5 commercial muskmelon varieties, commonly grown in California, to Verticillium wilt under greenhouse conditions*

Variety	Total number of plants	Number dead or diseased	Percentage diseased
Casaba .....	137	126	91.7
Hale's Best .....	154	120	77.8
Honey Ball .....	124	67	54.0
Honey Dew .....	150	122	81.3
Persian .....	135	119	88.1

The results of susceptibility trials presented in table 1 show that all of the 5 main crop-melon varieties grown in California, including Hale's Best and Honey Dew, are susceptible to *Verticillium* wilt and, with the exception of Honey Ball, no material difference in susceptibility was evident. Honey Ball, while showing considerably less disease than the other 4 varieties, was in no sense immune.

#### SUMMARY

A destructive *Verticillium* wilt occurred in a large planting of Persian muskmelons in California in 1932. The varieties Hale's Best and Honey Dew, growing in the same field, did not show the disease. Greenhouse tests established the pathogenicity of the *Verticillium* in question and also showed that, under greenhouse conditions, the varieties Casaba, Hale's Best, Honey Ball, Honey Dew and Persian are susceptible to the disease.

# BIG VEIN, A DISEASE OF LETTUCE

IVAN C. JAGGER AND NORMAN CHANDLER

(Accepted for publication March 14, 1934)

In 1922 an investigation of brown blight of lettuce<sup>1</sup> was begun in the Imperial Valley, California. This disease causes a pronounced stunting and gradual dying of plants, accompanied by characteristic yellow, discolored spots in younger leaves and brown, dead, irregular blotches and streaks in older ones. From the first, and often associated with characteristic brown blighted plants, occasional plants were observed that showed very striking, enlarged light yellow leaf veins (Fig. 1, A). It was at first thought that this striking symptom might be another phase of brown blight, but it now seems probable that big vein is a disease wholly distinct from brown blight.

Big vein is not apparent on lettuce until after the seedlings have 5 or 6 leaves, when plants are attacked in all stages of growth. The first noticeable symptom is a slight yellowing along the veins. This gradually becomes more pronounced, accompanied by considerable thickening of the whole leaf and more or less crinkling, until the pronounced symptoms shown in figure 1 are evident. All leaves of affected plants show symptoms more or less characteristic of a systemic disease. There are never any indications of plants being killed or of dead or brown tissue in any part of the plants. Symptoms are most pronounced in actively growing plants sometime before maturity. When the heads have reached maximum firmness and are ready for harvesting, the symptoms usually are less pronounced, for the yellow veins become somewhat green and there is a normal fading of interlying green leaf areas at maturity. In the winter-lettuce sections, in crops maturing during the cooler weather of midwinter, big-vein symptoms are usually more pronounced, and there are possibly higher percentages of affected plants than in crops maturing during the relatively warmer weather of late fall and early winter. Affected plants continue to grow and to head, but the heads are reduced to about half size and are of doubtful quality and value.

There has been a slow increase in the occurrence of big vein in the Imperial Valley from the time it was first observed. About 1929 it began to cause appreciable injury in a few fields and to attract the attention of growers. During the past few years it has become necessary to discontinue

<sup>1</sup> Jagger, Ivan C. Lettuce breeding for disease resistance progresses rapidly. U. S. Dept. Agr. Yearbook 1931: 348-350. 1931.



FIG. 1. Iceberg type lettuce A Young plant affected with big vein B Healthy young plant C Leaf affected with big vein. D Healthy leaf.

lettuce on a very limited acreage of soil where several consecutive crops of it have been grown and upon which lettuce has become seriously affected. But the disease is not yet seriously interfering with the lettuce industry of the Valley as a whole. For several years occasional typical big-vein specimens have been found in the Salinas-Watsonville lettuce section and in

other smaller sections in that part of California. Although there is probably a slow increase in the disease from year to year, no commercial injury to crops has so far been recorded in those sections. During the past 2 or 3 seasons it has been noted in the principal lettuce sections of Arizona. The disease is largely confined at present to heavier, naturally poorly drained soils, and, so far as known, it occurs only in California and Arizona.

Big vein is a soil-borne disease, which increases from year to year in favorable types of soil under constant lettuce culture. In pot cultures it has never attacked plants in containers of noninfested soil, although surrounded with containers of infested soil in which all plants showed big veins. New lettuce land, separated from old, severely infested lettuce land by only a roadway or a ditch, usually shows only a trace of the disease the first year. Big vein is apparently not readily transmitted except through soil. The application of various chemicals and fertilizers to infested soil, such as manganese sulphate, zinc sulphate, boric acid, iron sulphate, copper sulphate, various commercial fertilizers, stable manure and green cover crops, has not reduced the disease. In pot cultures a considerable percentage of the soluble salts have been leached out of infested soils without affecting the disease. Partial sterilization of the soil with either steam or formaldehyde eliminates it. Symptoms and behavior of big vein indicate that it may be similar to the soil-borne mosaic of wheat,<sup>2</sup> although no definite proof of its being caused by a virus has yet been obtained.

At present the only method of avoiding losses from big vein is to discontinue lettuce on land that has become sufficiently infested to cause appreciable injury to the crop. Thus far, it has become destructive only where several crops of lettuce have been grown in succession, and it is possible that a relatively short rotation with other crops may control it. Definite information on this point, however, is not yet available. Considerable effort has been made to obtain varieties or strains of lettuce that are resistant to the disease, but very little progress has been made.

Big vein and brown blight have the same general distribution, but in several smaller lettuce areas only one or the other has been found. In areas where both occur many fields show both, but in others either may occur alone. The occurrence of brown blight is related to the same soil conditions as described above for big vein, and it is possible that the two diseases are of similar nature. Brown blight, however, increases in severity and spreads much more rapidly than big vein, on a wide range of soil types and without responding readily to control by crop rotation. Brown blight had attained alarming prevalence and severity, threatening the lettuce industry of California and Arizona with destruction prior to its being brought under com-

<sup>2</sup> McKinney, H. H. A mosaic disease of winter wheat and winter rye. U. S. Dept. Agr. Bull. 1361. 1925.

plete control by essentially immune strains of lettuce that have been developed by the writers during the past few years. Before brown-blight-resistant strains were available, much soil in California and Arizona became so severely infested with the disease after producing 2 or 3 crops of lettuce that it was necessary to discontinue its culture. Under such conditions the less virulent big vein seldom became prevalent, but now the growing of brown-blight-resistant strains for several years without rotation apparently is causing certain [permitting favorable] soils to become heavily infested with the big-vein disease. In every way big vein appears to be a less serious disease than brown blight, but it may become generally troublesome, at least on the heavy soils of California and Arizona, if definite methods of control are not found.

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# INHERITANCE OF RESISTANCE TO POWDERY MILDEW, ERYSIPHE GRAMINIS TRITICI, IN WHEAT

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(Accepted for publication January 11, 1934)

In a previous publication,<sup>2</sup> it has been shown that a number of varieties of wheat possess more or less resistance to the *tritici* race of *Erysiphe graminis* DC. The varieties Axminster (C.I. 1839),<sup>3</sup> Chul (C.I. 2227), Dixon (C.I. 6295), Huron (C.I. 3315), and several selections of Illinois No. 1, Norka (C.I. 4377), Red Fern, and Sonora (C.I. 4293) were outstanding among common wheats for their marked resistance to physiologic form 1. To this physiologic form of the mildew they showed few or no signs of infection in the seedling stage when inoculated with conidia. All except Norka and Axminster were similarly highly resistant to physiologic form 2. In marked contrast they showed pronounced susceptibility to physiologic form 2.

## RESISTANCE-INHERITANCE STUDIES

In 1928, attention was first directed to a study of the inheritance of resistance. Crosses were made between a number of the resistant varieties and various susceptibility wheats in 1928, 1929, and 1930. The first generations were grown in the field. The second generations were for the most part planted in the greenhouse and inoculated in the seedling stage (3-4 leaf). This was easily and thoroughly accomplished by developing the mildew on a susceptible variety. When the mildew was sporulating abundantly, pots of the infected plants were shaken over the seedlings to be studied. These were then atomized with a fine spray of water and covered for 24 hours with wet muslin cloths. The resulting infection was abundant and usually uniform. In 10 to 14 days, the infection had reached a development so that seedlings could be classified for their mildew reaction. In most cases inoculated seedlings were not grown to maturity. In instances where the third generation was studied a portion of the second generation was spaced in the field and each plant harvested separately. about 15-25 seedlings of each of the lines of the third generation were then inoculated and studied in the greenhouse.

<sup>1</sup> This study was started while the writer was a member of the Department of Botany, Purdue Agricultural Experiment Station, and of the Division of Cereal Crops and Diseases of the United States Department of Agriculture. It has been continued at the University of Michigan. Papers of the Department of Botany and the Herbarium of the University of Michigan. No. 458.

<sup>2</sup> MAINS E. B. Host specialization of *Erysiphe graminis tritici*. Proc. Nat. Acad. Sci. 19: 49-53. 1933.

<sup>3</sup> Accession numbers of the Division of Cereal Crops and Diseases.

1. *Inheritance of Resistance of Norka to Mildew.* Until physiologic form 2 of the mildew was discovered in 1932, Norka was one of the most outstanding varieties for resistance. Throughout 5 years of previous study it showed no signs of infection beyond occasional faint flecking, although repeatedly inoculated.

For this study Norka was crossed with Webster (C.I. 3780), Ceres (C.I. 6900), Chinese (C.I. 6223), Kota (C.I. 5878), Malakoff (C.I. 4898), Reliance (C.I. 7370), and Warden (C.I. 4994), varieties that were found to be very susceptible to powdery mildew in the seedling stage. In the second generation the progenies segregated into 2 very distinct classes. One was highly susceptible like the susceptible parent. In marked contrast, the other showed little or no evidence of infection. While most of the seedlings of this group were fully as resistant as the Norka parent, some showed a very slight development of mildew. Table 1 gives the results obtained from a study of the second generation of these crosses.

It is evident in all of the crosses that the resistance of Norka segregated in a ratio closely approximating 3 resistant to 1 susceptible.

Two of the crosses were studied in the third generation. In the cross Norka  $\times$  Chinese, 662  $F_3$  lines were inoculated. Of these 160 proved to be uniformly resistant, 349 contained resistant and susceptible individuals, and 153 were uniformly susceptible. This is a very close approximation to the 1:2:1 ratio of 165.5:331:165.5 expected, on the basis of a single genetical factor. The 349 segregating lines gave a total of 14,062 individuals. Of these 10,563 were resistant and 3,499 susceptible, giving a ratio of 3.005: .995.

TABLE 1.—*Inheritance of the resistance of Norka to Erysiphe graminis tritici p.f.1. Segregation in  $F_2$*

Susceptible parent	Resistant segregates	Susceptible segregates	Ratio	Dev.	P.E.	$\frac{P.E.}{Dev.}$
Webster .....	654	213	3.02: 0.98	3.75	8.60	0.44
Ceres .. .....	1346	488	2.94: 1.06	29.50	12.50	2.36
Chinese .....	446	158	2.96: 1.04	7.00	7.18	0.97
Kota ... ..	133	50	2.91: 1.09	4.25	3.95	1.09
Malakoff .....	592	191	3.03: 0.97	4.75	8.17	0.58
Reliance .....	117	41	2.96: 1.04	1.50	3.67	0.41
Warden .....	1173	366	2.95: 1.05	18.75	11.46	1.64

In the cross Norka  $\times$  Reliance, 136  $F_3$  lines were inoculated. Of these 29 were uniformly susceptible, 74 segregated into resistant and susceptible groups, and 33 were uniformly susceptible. This is a very close approximation to the expected 1:2:1 ratio of 34:68:34. In the 74 lines segre-

gating for resistance and susceptibility, a total of 2,729 individuals were studied. Of these 2,022 were resistant and 707 were susceptible, giving a ratio of 2.96:1.04.

It is evident from the data presented that the resistance of Norka to physiologic form 1 is inherited as a simple dominant Mendelian factor.

2. *Inheritance of Resistance of Norka to Mildew in Relation to Inheritance of Resistance to Leaf Rust.* Norka also has shown pronounced resistance to a number of the physiologic forms of the leaf rust of wheat *Puccinia rubigo-vera tritici* (*Puccinia trititica* Eriks.).<sup>4</sup> Since Norka is highly resistant to physiologic form 3 of this rust, the second generation of the cross Norka  $\times$  Ceres was studied for inheritance of the resistance of Norka to both p.f.1 of mildew and p.f.3 of leaf rust, Ceres being susceptible to both. This was done by inoculating with both mildew and rust simultaneously. Plants susceptible to the rust produced uredinia, while those resistant showed only faint flecking or occasionally small uredinia.

A little difficulty was experienced in classifying the reaction of some of the plants to rust due to the peculiar effect of mildew on rust reaction. As had been noted a number of times previously in the leaf-rust investigations, when a variety of wheat highly resistant to leaf rust became infected with both mildew and rust rather large uredinia of the rust would develop in places where the mycelia of the two fungi intermixed.<sup>5</sup> In portions of the leaf tissue where the mycelia of the mildew were not present no uredinia and only flecks developed. Apparently, the cells of the host invaded by the mildew were more favorable for the development of the mycelia of the rust than uninfected cells. Although this makes some difficulty in classifying the rust-resistant individuals, by careful examination of such plants in areas uninfected by the mildew they can be properly placed.

Out of 1,834 individuals studied for reaction to physiologic form 3 of leaf rust 1,409 were resistant and 425 susceptible. This gives a ratio of 3.07:0.93, closely approximating a 3:1 ratio. The deviation is 33.5 and P.E. 12.50, the deviation  $\div$  P.E. equaling 2.68. This indicates that resistance of Norka to p.f.3 of leaf rust is due to a simple Mendelian factor. According to their reactions to both rust and mildew the individuals were classified into four groups. Of the 1,834 plants, 106 were susceptible to both rust and mildew, 319 were susceptible to rust and resistant to mildew, 382 were resistant to rust and susceptible to mildew, and 1,027 were resistant to both rust and mildew. This gives a ratio of .92:2.78:3.33:8.97. These results agree closely with those expected for the segregation of 2 independent factors.

<sup>4</sup> MAINS, E. B., and H. S. JACKSON. Physiologic specialization in the leaf rust of wheat, *Puccinia trititica* Eriks. *Phytopath.* 16: 89-120. 1926.

<sup>5</sup> See also discussion of this phenomenon by Johnston, C. O., *Phytopath.* 24: 1045-1046. 1934.

3. *Inheritance of Resistance of Red Fern to Mildew.* Red Fern has shown a very high resistance to both physiologic forms of powdery mildew throughout the 6 years of study. This variety was crossed with the susceptible Chinese (C.I. 6223), and 3,333 plants of the second generation were studied. These were separated into a resistant group giving a reaction similar to the Red Fern parent and a susceptible group having a reaction similar to Chinese. The segregation was 2,508 resistant to 825 susceptible, giving a ratio of 3.01 : .99 a Dev. of 8.25, P.E. 16.87. The resistance of Red Fern is, therefore, also due to a simple dominant Mendelian factor.

4. *Inheritance of Resistance of Hope (C.I. 8178).* The resistance of Hope to physiologic form 1 was somewhat more difficult to study than that of the previous varieties discussed. Its reaction is more or less variable. It usually gives a 0 to 1 reaction, but occasionally may reach about 2+. The most resistant reaction was produced during midwinter and the least in late spring. In order to study segregation with any degree of accuracy it is desirable to make the inoculations during midwinter. Even so the separation into classes in this cross was much more difficult than in those with Norka and Red Fern. Plants showing reactions of 0-2 were classed as resistant and those having reactions of 3-4 were classed as susceptible. The results of these studies are given in table 2.

TABLE 2.—*Inheritance of the resistance of Hope to Erysiphe graminis tritici p.f.1. Segregation in F*

Susceptible parent	Resistant segregates	Susceptible segregates	Ratio	Dev.	P.E.	Dev. P.E.
Chinese	89	302	0.91: 3.09	8.75	5.78	1.51
Marquis	45	112	1.15: 2.85	5.75	3.66	1.57
Michigan Amber	83	287	0.90: 3.10	9.50	5.62	1.69
Reliance	125	420	0.92: 3.08	11.25	6.82	1.65

The third generation was studied in the case of 2 crosses. In the cross Hope  $\times$  Chinese, 205  $F_3$  lines were studied. Of these, 51 proved to be uniformly resistant, 106 showed segregation, and 48 were uniformly susceptible. This is very close to the expected 1:2:1 ratio, which would be 51:102:51. The 106 segregating lines produced 868 resistant and 2,515 susceptible individuals, a ratio of 1.03:2.97.

For the cross Hope  $\times$  Marquis 236  $F_3$  lines were studied. Of these, 59 were uniformly resistant, 122 segregated and 55 were uniformly susceptible. This also is very close to the expected 1:2:1 ratio which would be 59:118:59. The 122 segregating lines produced a total of 1,315 resistant

individuals and 3,636 susceptible, giving a ratio of 1.06 : 2.94. Apparently, the resistance of Hope to physiologic form 1 is due to a single recessive factor.

5. *Inheritance of Resistance of Sonora.* The resistance of Sonora to physiologic form 1 was studied in a cross with Chinese. Of the 131 plants of the  $F_2$ , 38 were highly resistant and 93 were very susceptible. This is a ratio of 1.16 : 2.84 with a Dev. of 5.25 and P.E. of 3.34. Apparently, the resistance of Sonora is recessive and also due to a single factor.

6. *Inheritance of Resistance of Michigan Amber 29-1-1-1.* The reaction of Michigan Amber 29-1-1-1 to p.f.1 is very unusual. Of all the varieties studied, it has shown the greatest variability. In the seedling stage in the greenhouse, during the winter months, it was usually 0 to 1. Under more favorable growing conditions for the variety it was much more susceptible, even giving a reaction of 3. When this reaction was first encountered early in the fall, it was very striking in contrast with the marked resistance previously noted for the variety, and it was thought that a new physiological form had been found. Inoculations of Michigan Amber 29-1-1-1, later in the season, showed that it was a variation in the reaction of the variety. This condition made a study of the inheritance of the resistance especially difficult. The first attempt made late in the winter showed no sharp separation into classes, so that no conclusions were possible. However, a population of 297 plants of the  $F_2$  of a cross between Michigan Amber 29-1-1-1 and Chinese (C.I. 6223) was studied earlier the succeeding winter when Michigan Amber was showing pronounced resistance. This segregated into 81 resistant (0-2) and 216 susceptible (3-4), a ratio of 1.09 : 2.91 with a Dev. of 6.75 and P.E. of 5.03. This would indicate that the resistance manifested in the seedling stage is probably inherited as a recessive simple factor. Further study with the variety would seem to be desirable.

#### SUMMARY

The resistance of Norka (C.I. 4377) in the seedling stage to physiologic form 1 of *Erysiphe graminis tritici* is inherited as a simple dominant factor. The same is true for the resistance of Red Fern.

The resistance of Hope (C.I. 8178) in the seedling stage is inherited as a simple recessive factor. The same is true for the resistance of Sonora (C.I. 4293) and apparently also for Michigan Amber 29-1-1-1 under conditions favorable for its maximum expression.

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# THE RESISTANCE OF SAPLINGS AND CERTAIN SEEDLINGS OF *PINUS PALUSTRIS* TO *SEPTORIA ACICOLA*<sup>1</sup>

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(Accepted for publication February 6, 1934)

Before vigorous height growth has started, most seedlings of *Pinus palustris* Mill. are very susceptible to attack by *Septoria acicola* (Thum.) Sacc., the pathogen of the brown-spot needle blight,<sup>2,3</sup> the fungus readily advancing in the needles to cause large dead areas by the end of the first season of the needles. During the spring of the second season the advance is so fast in severely infected seedlings, especially when grass and other ground cover are scarce around the seedlings, that typical brown spots are not formed but whole needles yellow and die below the old brown spots. After vigorous height growth starts, and with certain individual seedlings before it starts, a marked resistance to the fungus is evident, the brown spots being small and definitely limited, even through the second growing season of the needles.

Associated with typical brown-spot lesions on resistant seedlings, saplings, and to a less extent on mature trees, are "bar-spots," consisting of a small area surrounded by a yellow zone that may extend from less than 1 to 3 mm. from the brown area. Bar-spots have but a sparse mycelium in the mesophyll layer, have many resin-filled cells, and only very rarely possess the fruit bodies of *Septoria*. They seem to indicate resistance, the host reacting to prevent the normal growth and reproduction of the fungus. Bar-spots have been found on *Pinus caribaea*, *P. pinaster*, *P. glabra*, *P. taeda*, and *P. echinata*, in addition to *P. palustris*.

During the period of January to July, 1933, a number of observations and experiments were made near Bogalusa, Louisiana, to determine the nature of this resistance.

Saplings 6 to 10 feet high, bent over so that the terminal buds opened within a few inches of abundantly infected seedlings, developed only 0.5 per cent dead leaf length by June 23, while the near by seedlings showed about 15 per cent at the same time, indicating that the resistance of sap-

<sup>1</sup> The investigations here reported were made by the writer while employed as Field Aid by the Division of Forest Pathology, Bureau of Plant Industry, in cooperation with the Southern Forest Experiment Station, U. S. Forest Service. Acknowledgment is due Mr. P. V. Siggers of the Division of Forest Pathology for much assistance.

<sup>2</sup> Hedgcock, Geo. G. *Septoria acicola* and the brown-spot disease of pine needles. *Phytopath.* 19: 993-999. 1929.

<sup>3</sup> Siggers, Paul V. The brown-spot needle blight of longleaf pine seedlings. *Jour. For.* 30: 579-593. 1932.

lings is real. There seems to be a change from susceptibility to resistance with the initiation of vigorous height growth.

The resistance of certain seedlings is likewise real. An almost disease-free seedling may be growing beside one with a high degree of infection. Both may be growing under the same conditions of soil and ground cover, and the susceptible plant may be smaller or larger and with fewer or more leaf fascicles than the diseased one.

The anatomical differences between the leaves of saplings and seedlings and of resistant and susceptible seedlings are, in general, independent of the amount of dead leaf length and are determined chiefly by the degree of shade in which the leaves developed. Stomata are sufficiently abundant on all to insure avenues of entrance for the pathogen. However, seedlings often times have but 2 resin ducts in a needle, while saplings usually have 3 or 4. Susceptible and resistant seedlings do not differ from each other in this respect.

The difference between the number of resin ducts of saplings and seedlings suggested that resin production may have some bearing on resistance. Diseased spots were dissected from needles and the resin extracted with an excess of ether. The samples were dried for 2 hours at 100° C. and weighed, both before and after extraction, and the difference between the 2 weights taken as the amount of resin extracted. Each sample was composed of spots from a large number of plants and the percentage of resin based on the dry weight of the sample before extraction.

The brown spots from seedling and sapling needles yielded 9.33 and 16.46 per cent resin, respectively, while the bar-spots from saplings, *i.e.*, the type of infection in which the fungus had made the least headway, yielded 20.89 per cent.

A second set of samples was extracted with xylol at 80° C. and showed 7.11 and 13.90 per cent resin in brown spots of seedlings and saplings, respectively. Xylol extractions of spots on definitely susceptible and definitely resistant seedlings showed 6.16 per cent resin in the former and 11.60 per cent in the latter.

The same difference in the ability to produce resin can be shown by cutting off the top halves of needles of nearly disease-free seedlings and saplings and extracting the resin that exudes and hardens on the cut surfaces. Five days after cutting, xylol extractions of the cut surfaces ( $\frac{1}{8}$ -in. sections back from the cut) showed that saplings produce 23.60 and seedlings 13.69 per cent. resin.

In another and later set, resistant and susceptible seedlings exuded 22.84 and 15.71 per cent resin, respectively.

Bar-spot infection is more common on slash pine, *Pinus caribaea*, and on longleaf pine, *P. palustris*, than on shortleaf pine, *P. echinata*, and loblolly

pine, *P. taeda*. The first two are the highest resin producers and are the trees commonly used as American producers of turpentine. The tree employed for this purpose in Europe, *P. pinaster* Ait., seems to produce mostly bar-spots, as far as can be determined from the few samples present near Bogalusa, Louisiana. Thus, bar-spots, in which the fungus makes the least headway, are more common on the hosts that have the greatest ability to produce resin.

#### CONCLUSION

All the evidence indicates that one of the factors responsible for the resistance of saplings and certain seedlings of *Pinus palustris* to the brown-spot fungus, *Septoria acicola*, is related to the ability of the host to produce resin and thus limit the extension of the fungus within the host. This inhibiting influence of the resin may be due either to a toxic effect or, more likely, to a change in the moisture conditions, or to the resin's acting as a mechanical barrier.

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# SEED TRANSMISSION OF VERTICILLIUM WILT OF EGGPLANTS AND TOMATOES

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(Accepted for publication March 9, 1934)

Although wilts caused by *Verticillium* spp. have been studied by a great many workers and have been reported from numerous host plants (3), so far as the writer is aware there are in the literature only 2 references to seeds as possible carriers. Wollenweber (5) isolated *Verticillium* sp. from wheat seed in Germany, but did not establish its pathogenicity. Richardson (2) planted seeds of the eggplant that showed vascular discoloration and had 6 plants die of typical *Verticillium* wilt. He did not eliminate the possibility of infection by soil-borne *Verticillium*. He also mentions a seed isolation but gives no details of the technic followed. Since proof of seed transmission of *Verticillium* wilt is not altogether conclusive, publication of the following investigation seems timely.

*Verticillium* wilt of eggplant usually is attributed to *Verticillium albo-atrum* R. & B. (4), but Richardson (2) regards the causal fungus as *Verticillium Dahliae* Kleb. He bases his conclusion upon the presence of pseudosclerotia in culture. *Verticillium*-wilt fungi, with and without pseudosclerotia, have been isolated from eggplants by the writer, although in the great majority of cases they were present.

To determine whether or not the wilt fungus is carried by the seeds of eggplant and tomato, the problem was divided into external and internal carriage. To study external carriage, seeds were aseptically removed from eggplants and tomatoes that showed definite vascular discoloration in the fruits. Some were plated in Petri dishes on 2 per cent potato-dextrose agar immediately upon removal, while others were placed in sterile flasks and allowed to dry at room temperature for 2 months before plating. The results are given in table 1.

TABLE 1.—*Ferticillium* infestation of the seeds of eggplant and tomato

Number of seeds	Kind of seeds	Time of plating	Results <sup>a</sup>
100	Eggplant	At removal from berry.	54 +
100	Eggplant	Two months after removal	39 +
25	Tomato	At removal from berry	10 +
25	Tomato	Two months after removal	14 +

<sup>a</sup> Plus (+) indicates that the fungus was identified as being culturally identical to that isolated from stems of wilted eggplants and tomatoes.

A portion of the eggplant seeds that had been aseptically removed to sterile flasks and then allowed to dry for 2 months was shaken for 15 minutes in sterile water. A poured-plate series of potato-dextrose agar containing 2 cc. of the wash water was made. After 6 days an examination of the 25 poured plates revealed 7 typical colonies of *Verticillium*. About 200 seeds were involved in this test.

Possible internal seed infection seemed the most important aspect of the problem, since, in its presence, seed-sterilization methods would necessarily

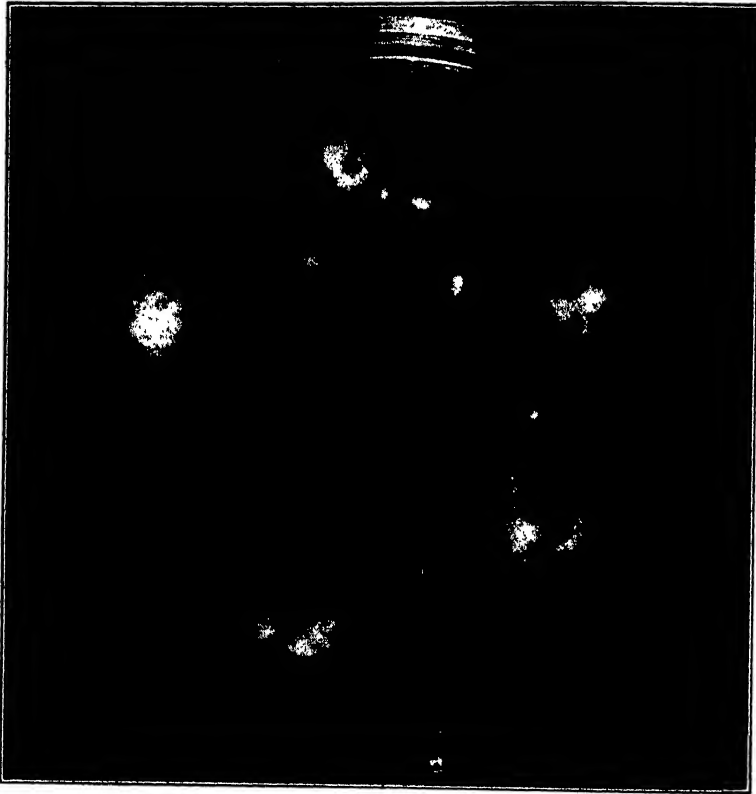


FIG. 1. *Verticillium* isolated from interior of eggplant seed.

be limited. Seeds for this study were secured from fruits with discolored bundles, but were separated from the fleshy part of the fruit by the usual fermentation method. One group of seeds used in the test were taken at random from commercial packets. The method of sterilization was principally the same as that used by the writer and L. K. Jones (1) when studying seed transmission of *Fusarium* wilt of peas. In brief, 1 gram of calcium hypochlorite is used to every 14 cc. of sterile distilled water. The

mixture is stirred frequently and after 15 minutes is filtered. The seeds to be treated are allowed to stand with frequent stirring for  $1\frac{1}{2}$  to 2 hours in about 5 times their volume of the filtrate. (Since the amount of available chlorine varies considerably with different lots of calcium hypochlorite, the exact time for sterilization without injuring the seeds to be treated must be determined by actual trial.) After treatment they were removed to 2 per cent potato-dextrose agar and allowed sufficient time to develop. To establish the toxicity of this treatment to the wilt fungus, a culture containing pseudosclerotia was placed in a portion of the filtrate, and, after intervals of one-half hour, parts of it were washed thoroughly in sterile distilled water and plated on 2 per cent potato-dextrose agar. All treatments including the  $\frac{1}{2}$ -hr. treatment were toxic. Similar bits of the fungus without treatment, but washed in distilled water and plated, grew readily. Figure 1 shows a plate of eggplant seeds treated as above with an unusually high percentage of seed infection.

The pathogenicity of typical cultures was established by inoculating sterilized soil with plate cultures and planting it to eggplant from seeds especially selected from wilt-free plants. Checks were grown on sterilized soil from the seeds similarly selected. The checks remained healthy, while those in the inoculated soil showed the typical wilt. Reisolations showed the fungus to be culturally identical to that used for inoculation. Table 2 gives the results of the internal seed carriage tests.

TABLE 2.—*Verticillium-infection of seeds of eggplant and tomato*

Number of seeds	Kind of seeds	Sources of seeds	Results
100	Eggplant	Diseased fruit	31 +
100	"	" "	24 +
200	"	Commercial packets	3 +
25	Tomato	Diseased fruit	11 +

It is to be remembered that all the seeds, except those of one commercial lot, were specially selected and, therefore, probably represent a much greater percentage of infected seed than would generally be encountered. It does not necessarily follow, however, that certain lots of commercial seeds could not be as badly infected. Many cases have been observed in Illinois where the eggplant appeared perfectly healthy, but an examination of the fruits thereon showed some vascular discoloration. Isolations from such fruits gave typical *Verticillium* cultures.

In limited tests of eggplant seeds the hot-water treatment at 120° F. for 20 minutes seemed to control the disease. Because of a shortage of seeds, hot-water tests were not run with tomatoes, and, for the same reason,

only a limited number of tests were made of eggplants. A test-tube culture of the fungus containing pseudosclerotia was not killed by a similar treatment. The pseudosclerotia, however, seemed to be the only portion of the culture to survive.

Seed separation by the usual fermentation method did not seem materially to reduce the amount of seed infection in either eggplants or tomatoes. Selection of clean seed is highly recommended.

#### SUMMARY AND CONCLUSIONS

1. The *Verticillium*-wilt fungus may be carried by tomato and eggplant seeds. Internal carriage seems to be the most important method and may account for the apparent coexistence of the disease with eggplant culture in Illinois. At the present time, the extent of *Verticillium* wilt of tomatoes in this State is not known.

2. A hot-water treatment at 120° F. for 20 minutes may satisfactorily sterilize infected seeds of eggplant, but this point is not definitely established. Selection of disease free seed is highly recommended.

DEPARTMENT OF HORTICULTURE

ILLINOIS AGRICULTURAL EXPERIMENT STATION

URBANA, ILL.

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# CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

## II. THE PROTEOLYTIC ACTION OF PEPSIN

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### INTRODUCTION

Experiments with tobacco-mosaic virus and trypsin already described (8) showed that certain host plants were not susceptible to virus in the presence of trypsin, and that this was due chiefly to an effect of enzyme on the test plant and not, as had been thought previously (6), to proteolytic action of enzyme on virus. Consequently, no evidence as to whether or not the virus is a protein was obtained. However, if the virus of tobacco mosaic is a protein, it seems likely that enzymatic hydrolysis might occur on exposure to some other proteolytic enzyme. The demonstration of such proteolytic hydrolysis, and more particularly of a rate of hydrolysis, would be of interest since it would indicate that the virus is a protein or very closely associated with a protein. The enzyme pepsin was selected for study in this connection because its proteolytic activity extends over a very wide range of substrates and because it was available in a very pure form. Previous workers have, of necessity, used pepsin in the presence of other enzymes, since, until recently, it has been available only in mixtures. Flexner, Clark, and Dochez (2) studied the effect of placing the virus of poliomyelitis in the stomach of monkeys, thus subjecting it to the action of pepsin and other enzymes, and found that such treatment did not inactivate the virus. Lojkin and Vinson (6) reported that pepsin had no action on tobacco-mosaic virus, and Caldwell (1) found that pepsin had no effect on aucuba-mosaic virus. The present paper records the results obtained in a study of the proteolytic action of pepsin on the virus of tobacco mosaic.

### MATERIALS AND METHODS

The virus used in the experiments to be reported consisted of untreated infectious juice and a purified virus preparation. The untreated infectious juice was obtained by pressing plants of mosaic-diseased *Nicotiana tabacum* L. variety Turkish that had been grown in a greenhouse in winter. The plants were cut and kept in a freezing room for a few days and then allowed to thaw just before pressing. The juice was filtered through two layers of bandage gauze and then diluted with two volumes of distilled water. The purified virus was prepared from infectious juice by pre-

<sup>1</sup> Published at the expense of The Rockefeller Institute for Medical Research out of the order determined by the date of acceptance of the manuscript.

precipitation with lead acetate and elution with 0.1 M potassium phosphate at pH 7. The procedure described by Vinson and Petre (9) was modified somewhat for the preparation of purified virus used in this work. The purified virus was diluted with 9 parts of 0.1 M potassium phosphate at pH 7, so that its infectivity would be comparable to that of the diluted untreated infectious juice described above.

Commercially available Parke-Davis pepsin U. S. P. 1:10,000, and a specially prepared crystalline pepsin<sup>2</sup> were used. Commercial pepsin, which is a mixture of pepsin with small amounts of other enzymes (7), was used in the form of a dry powder, added directly to the virus preparations. The crystalline pepsin, which is believed to be only one enzyme (7), was used in the form of a solution in 0.02 N HCl containing 10 mg. of pepsin nitrogen per cc.

Portions of the virus preparations were taken to pH 3, 4, 5, 6, 7, and 8 by the addition, with vigorous stirring, of 2 N HCl or NaOH. Pepsin was added to portions of each of the above solutions. Due to the fact that the virus preparations were well buffered, the addition of pepsin caused but little change in the hydrogen-ion concentration of the solutions. This change was never over 0.3 pH unit and usually less than 0.1 pH unit. After the addition of pepsin, the solutions were immediately tested for virus, using *Nicotiana glutinosa* L. and in certain instances *Phaseolus vulgaris* L., and then incubated at 37° C. for 97 days. The solutions were tested for virus infectivity 6 times during the incubation period in order to follow the course of the reaction. These tests were made by the half-leaf method, as previously described (8). Each of the virus-plus-pepsin preparations was tested against a control that consisted of another portion of the same virus preparation to which no pepsin had been added. In every instance, therefore, the control-virus preparation was exactly the same as the corresponding pepsin-virus preparation, except for the absence of pepsin. *N. glutinosa* was used as the test plant in the majority of the experiments, since its response to virus was found to vary but little over the range pH 3 to pH 8. In addition, *P. vulgaris* was used to test all solutions at pH 4 and pH 7.

#### EXPERIMENTAL

A 260 cc. portion of diluted purified virus was taken to pH 3 by the addition of 2 N HCl, with vigorous stirring, and a few drops of toluene were added. To 60 cc. of this preparation was added 60 mg. of commercial pepsin. The mixture was then divided into 3 equal portions. One 20 cc. portion was immediately frozen in 6 small test tubes and allowed to remain in a room held at -15° C. A control solution in 6 small test tubes also was

<sup>2</sup> The writer is indebted to Dr. Northrop and Dr. Herriott for this crystalline pepsin.

placed in this room. The remaining 2 portions were immediately tested for virus infectivity on *Nicotiana glutinosa* against the proper controls and then placed in a room at 37° C. To 2 other 20 cc. portions of the diluted purified virus at pH 3 were added 2 mg. and 200 mg. of commercial pepsin, respectively. These preparations were immediately tested for virus infectivity on *N. glutinosa* against the proper controls, and then placed in a room at 37° C. To other 20 cc. portions of diluted purified virus at pH 4, 5, 6, 7, and 8 were added 20 mg. of commercial pepsin, respectively. These preparations were likewise immediately tested for virus infectivity on *N. glutinosa* against controls at the different hydrogen-ion concentrations. The preparations at pH 4 and pH 7 were also tested on *Phaseolus vulgaris*. These preparations and controls for all the preparations described above were placed in a room at 37° C.

The above described procedure was repeated, using diluted untreated infectious juice in place of the diluted purified virus preparation. Then the procedures just described, involving the use of commercial pepsin with diluted purified virus, and with diluted untreated infectious juice, were repeated, using 0.01 cc. of a crystalline pepsin solution containing 10 mg. of pepsin nitrogen per cc. in place of each milligram of commercial pepsin. It will be seen that a study of the action of commercial pepsin at a concentration of 1 mg. per cc., and of crystalline pepsin at a concentration of 0.1 mg. of pepsin nitrogen per cc., on both diluted purified virus and diluted untreated infectious juice at pH 3, 4, 5, 6, 7, and 8 at 37° C., was included. The effects of increasing the pepsin concentration 10 times, of decreasing it to 1/10, and of carrying out the digestion at -15° C., were studied at pH 3. All preparations, except the frozen samples, were tested for virus infectivity on *Nicotiana glutinosa*, and in certain instances on *Phaseolus vulgaris*, immediately after addition of pepsin, and were then placed in a room at 37° C. After 2 days, portions of all preparations, including the frozen samples, were removed and again tested for virus. This was repeated at the end of 7, 14, 28, and 97 days. In addition, the 4 preparations containing the largest amount of pepsin were tested for virus at the end of 4 days.

A descriptive summary of the 40 different preparations of virus plus pepsin, including the hydrogen-ion and pepsin concentration of each, whether incubated or frozen, and the results of the tests for virus infectivity at the end of the various time periods, is given in tables 1, 2, 3, and 4. The average of the actual number of lesions per half-leaf for each preparation at the end of each period of digestion is given in the rows labeled "Actual." A number, which represents the quotient of the average number of lesions per half-leaf obtained with the virus-plus-pepsin preparation, divided by the average number of lesions per half-leaf obtained on the other halves of the same leaves with the control preparation, multiplied by 100,

TABLE 1.—The action of commercial pepsin on diluted purified tobacco-mosaic virus

Temperature of incubation	pH of solution	Mg. pepsin per cc.	Test plant	Tested →	Immediately	After 2 days	After 4 days	After 7 days	After 14 days	After 28 days	After 97 days
37° C.	3	10.0	<i>N. glutinosa</i>	Average <sup>a</sup> Actual <sup>b</sup>	94.7 56.5	17.7 7.2	1.7 0.5	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
37° C.	3	0.1	"	Average Actual	142.0 19.8	54.4 35.7	27.4 13.6	27.4 13.6	16.1 1.6	22.2 1.6	0.0 0.0
37° C.	3	1.0	"	Average Actual	156.0 37.5	87.1 14.9	24.6 2.1	24.6 2.1	9.3 0.0	0.0 0.0	0.0 0.0
37° C.	3	1.0	"	Average Actual	64.3 26.6	56.3 19.6	19.8 5.0	19.8 5.0	1.7 0.1	0.0 0.0	0.0 0.0
-15° C.	3	1.0	"	Average Actual	94.0 37.4	103.7 7.1	103.7 7.1	103.7 7.1	209.0 7.1	94.0 4.7	110.0 1.1
37° C.	4	1.0	"	Average Actual	143.0 11.7	148.0 16.2	81.6 24.4	81.6 24.4	55.0 9.5	44.2 3.8	23.0 3.9
37° C.	4	1.0	<i>P. vulgaris</i>	Average Actual	18.2 8.3	32.4 19.0	7.2 3.1	7.2 3.1	23.6 21.4	3.4 1.5	0.0 0.0
37° C.	5	1.0	<i>N. glutinosa</i>	Average Actual	100.0 15.2	86.5 15.2	99.4 29.2	99.4 29.2	109.5 20.4	90.6 9.7	133.0 10.8
37° C.	6	1.0	"	Average Actual	97.2 26.9	108.0 21.2	116.0 49.8	116.0 49.8	106.1 77.0	75.0 102.0	134.0 139.0
37° C.	7	1.0	"	Average Actual	132.0 44.8	72.0 19.4	100.0 27.5	100.0 27.5	77.0 33.0	102.0 42.6	139.0 51.0
37° C.	7	1.0	<i>P. vulgaris</i>	Average Actual	41.6 85.4	42.0 56.2	46.7 89.0	46.7 89.0	48.7 22.1	21.8 8.7	12.0 8.7
37° C.	8	1.0	<i>N. glutinosa</i>	Average Actual	114.0 36.0	89.0 18.5	98.8 7.7	98.8 7.7	70.0 6.0	155.0 8.2	110.2 8.4

<sup>a</sup> Numbers represent the average number of lesions per half-leaf obtained on 7 or more half-leaves of *Nicotiana glutinosa* or 16 or more half-leaves of *Phaseolus vulgaris* on inoculation with the designated virus-plus-pepsin preparation divided by the average number of lesions per half-leaf obtained on the other halves of the same leaves with the corresponding control preparation, multiplied by 100.

<sup>b</sup> Numbers represent the average of the actual number of lesions per half-leaf obtained on 7 or more half-leaves of *N. glutinosa* or 16 or more half-leaves of *P. vulgaris* on inoculation with the designated virus-plus-pepsin preparation.



TABLE 2.—The action of commercial pepsin on diluted untreated infectious juice

Tempera- ture of incubation	pH of solution	Mg. pepsin per cc.	Test plant	Tested →	Immedi- ately	After 2 days	After 4 days	After 7 days	After 14 days	After 28 days	After 97 days
37° C.	3	10.0	N. glutinosa	Average <sup>a</sup> Actual <sup>b</sup>	69.4 70.7	8.7 10.0	4.0 0.9	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
37° C.	3	0.1	"	Average Actual	130.0 14.5	110.0 23.0		35.1 23.9	17.7 6.0	35.0 2.6	16.0 1.0
37° C.	3	1.0	"	Average Actual	143.0 33.4	113.0 32.8		44.2 15.4	3.2 0.5	2.2 0.4	1.7 0.3
37° C.	3	1.0	"	Average Actual	112.0 55.8	68.5 24.4		30.6 17.9	0.0 0.0	0.0 0.0	0.0 0.0
-15° C.	3	1.0	"	Average Actual		101.0 26.9		124.7 43.7	208.0 16.2	163.0 23.2	147.0 12.2
37° C.	4	1.0	"	Average Actual	135.0 22.6	94.0 62.5		93.0 31.4	70.5 15.3	61.0 12.4	59.0 16.6
37° C.	4	1.0	P. vulgaris	Average Actual	26.6 3.6	45.2 14.3		36.4 4.1	16.0 6.5	11.5 3.6	11.0 1.0
37° C.	5	1.0	N. glutinosa	Average Actual	110.0 39.6	79.4 27.6		74.3 20.8	45.0 10.7	84.0 16.5	61.0 19.9
37° C.	6	1.0	"	Average Actual	45.2 26.1	66.1 27.0		81.9 34.0	78.2 20.3	91.0 20.3	65.2 35.5
37° C.	7	1.0	"	Average Actual	101.5 28.3	112.0 20.9		109.5 72.8	97.5 25.9	121.0 33.2	
37° C.	7	1.0	P. vulgaris	Average Actual	34.4 7.2	32.2 16.6		78.6 18.4	56.9 49.2	38.0 11.7	
37° C.	8	1.0	N. glutinosa	Average Actual	97.5 43.7	80.5 84.5		105.5 40.8	123.0 18.1	103.8 16.6	104.0 37.8

<sup>a</sup> Numbers represent the average number of lesions per half-leaf obtained on 7 or more half-leaves of *Nicotiana glutinosa* or 16 or more half-leaves of *Phaseolus vulgaris* on inoculation with the designated virus-pepsin preparation divided by the average number of lesions per half-leaf obtained on the other halves of the same leaves with the corresponding control preparation, multiplied by 100.

<sup>b</sup> Numbers represent the average of the actual number of lesions per half-leaf obtained on 7 or more half-leaves of *N. glutinosa* or 16 or more half-leaves of *P. vulgaris* on inoculation with the designated virus-pepsin preparation.

is also given for each preparation. This number, which is given in the rows labeled "Average," may be considered a measure of the virus present. It is recognized that the number may not be strictly proportional to the actual amount of virus. However, it serves as an indication of the amount of virus present and enables one to determine rapidly whether much, little, or no virus is left in the pepsin-treated solutions at the end of the various digestion periods. As long as this number remains near 100, it indicates that the pepsin-treated virus preparation produces about as many lesions as does the corresponding control preparation; hence much of the original virus must be present. As this number decreases, it indicates that a decreasing amount of virus is present in the pepsin-treated virus preparation. When no lesions are produced on inoculation of *Nicotiana glutinosa* or *Phaseolus vulgaris* with the pepsin-treated virus preparation, the number becomes zero.

The results given in columns headed by "At once" in tables 1 to 4 show that neither commercial nor crystalline pepsin has much immediate effect on the infectivity of either purified tobacco-mosaic virus or infectious juice over the range of pH 3 to pH 8, as measured on *Nicotiana glutinosa*. *Phaseolus vulgaris* was found to give erratic results, especially at pH 4. Because of previous work, it had been expected that the preparations at pH 4 or lower might give irregular results on *P. vulgaris*, but it was hoped that satisfactory results might be obtained at pH 7. It is obvious, however, that *P. vulgaris* is not a suitable test plant for the present experiments, since, even at pH 7, the susceptibility of the plants to virus is changed when pepsin is present. Consequently, in the following paragraphs, only the results obtained with *N. glutinosa* will be considered.

It may be seen further from tables 1, 2, 3, and 4 that neither 1 mg. commercial pepsin per cc. nor 0.1 mg. crystalline pepsin nitrogen per cc. has any appreciable effect on the infectivity of either purified tobacco-mosaic virus or diluted untreated infectious juice when incubated at 37° C. for several days at either pH 7 or pH 8. These pepsin-treated virus samples produced about as many lesions on inoculation as did the corresponding controls. When the hydrogen-ion concentration of the digestion mixtures was lowered to pH 5 or pH 6, there was a slight tendency for the infectivity of the virus-pepsin preparations to drop on prolonged digestion. This tendency became quite marked when the hydrogen-ion concentration of the digestion mixtures was further lowered to pH 4. At this hydrogen-ion concentration the pepsin-treated virus preparations produced only 2.1, 32.2, 23.0, and 59.0 per cent as many lesions on *Nicotiana glutinosa* after 97 days' incubation as did the corresponding controls. Furthermore, there was a definite trend to the reduction in infectivity, the pepsin-treated preparations, in general, producing a smaller percentage of lesions after each suc-

TABLE 3.—The action of crystalline pepsin on diluted purified tobacco-mosaic virus

Temperature of incubation	pH of solution	Mg. pepsin nitrogen per cc.	Test plant	Tested →	Immediately	After 2 days	After 4 days	After 7 days	After 14 days	After 28 days	After 97 days
37° C.	3	1.0	N. glutinosa	Average <sup>a</sup> Actual <sup>b</sup>	62.6 24.5	11.8 6.1	0.3 0.1	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
37° C.	3	0.01	"	Average Actual	93.5 15.9	53.4 32.4		32.8 17.2	17.8 0.8	2.5 0.4	2.7 0.3
37° C.	3	0.1	"	Average Actual	111.8 24.2	60.5 28.1		16.0 4.1	0.0 0.0	0.0 0.0	0.0 0.0
37° C.	3	0.1	"	Average Actual	88.4 52.0	46.0 15.4		9.1 2.5	0.0 0.0	0.0 0.0	0.0 0.0
-15° C.	3	0.1	"	Average Actual		73.5 12.1		91.0 13.0	170.0 4.6	93.0 4.0	84.5 1.6
37° C.	4	0.1	"	Average Actual	107.8 37.4	108.5 66.7		69.4 31.8	57.9 11.7	27.7 3.6	2.1 0.3
37° C.	4	0.1	P. vulgaris	Average Actual	48.0 18.5	24.6 19.6		29.8 15.2	48.3 76.4	9.5 3.0	0.1 0.1
37° C.	5	0.1	N. glutinosa	Average Actual	122.0 29.8	81.2 22.0		72.8 14.7	59.0 15.7	45.7 12.2	48.0 6.9
37° C.	6	0.1	"	Average Actual	85.2 35.0	129.0 42.5		85.0 37.8	65.0 20.6	95.5 28.3	88.0 34.2
37° C.	7	0.1	"	Average Actual	68.4 30.4	102.5 21.3		92.8 29.7	103.8 25.1	78.3 31.5	88.2 34.2
37° C.	7	0.1	P. vulgaris	Average Actual	56.5 101.0	101.0 149.0		107.1 134.4	78.6 146.1	62.0 108.5	59.5 64.2
37° C.	8	0.1	N. glutinosa	Average Actual	57.0 17.0	76.5 8.8		68.8 9.0	130.0 10.0	101.5 6.7	81.0 8.4

<sup>a</sup> Numbers represent the average number of lesions per half-leaf obtained on 7 or more half-leaves of *Nicotiana glutinosa* or 16 or more half-leaves of *Phaseolus vulgaris* on inoculation with the designated virus-plus-pepsin preparation divided by the average number of lesions per half-leaf obtained on the other halves of the same leaves with the corresponding control preparation, multiplied by 100.

<sup>b</sup> Numbers represent the average of the actual number of lesions per half-leaf obtained on 7 or more half-leaves of *N. glutinosa* or 16 or more half-leaves of *P. vulgaris* on inoculation with the designated virus-plus-pepsin preparation.

TABLE 4.—The action of crystalline pepsin on diluted untreated infectious juice

Tempera- ture of incubation	pH of solution	Mg. pepsin nitrogen per cc.	Test plant	Tested →	Immedi- ately	After 2 days	After 4 days	After 7 days	After 14 days	After 28 days	After 97 days
37° C.	3	1.0	<i>N. glutinosa</i>	Average <sup>a</sup> Actual <sup>b</sup>	77.3 37.6	15.8 14.5	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
37° C.	3	0.01	"	Average Actual	83.0 12.2	80.2 64.0	40.8 32.6	40.8 32.6	28.7 5.4	25.5 7.2	4.4 0.8
37° C.	3	0.1	"	Average Actual	111.0 64.3	68.3 25.3	24.8 14.4	24.8 14.4	0.0 0.0	0.0 0.0	0.0 0.0
37° C.	3	0.1	"	Average Actual	102.3 74.3	70.5 27.0	22.7 13.9	22.7 13.9	0.0 0.0	0.0 0.0	0.0 0.0
-15° C.	3	0.1	"	Average Actual	114.0 34.6	114.0 34.6	89.4 38.4	89.4 38.4	113.6 23.2	80.6 24.2	139.0 22.4
37° C.	4	0.1	"	Average Actual	92.5 38.6	74.5 53.3	70.0 42.0	70.0 42.0	63.1 37.6	67.8 27.0	32.2 10.5
37° C.	4	0.1	<i>P. vulgaris</i>	Average Actual	33.8 5.1	43.0 22.9	24.4 6.2	24.4 6.2	41.5 27.2	54.5 11.3	18.0 2.2
37° C.	5	0.1	<i>N. glutinosa</i>	Average Actual	90.5 26.4	57.0 49.2	69.5 53.1	69.5 53.1	116.6 38.3	99.3 24.4	66.0 26.9
37° C.	6	0.1	"	Average Actual	55.5 27.3	116.0 17.8	55.5 29.8	55.5 29.8	83.2 36.6	67.3 18.5	90.0 54.8
37° C.	7	0.1	"	Average Actual	79.8 41.5	123.0 34.4	108.0 69.3	108.0 69.3	99.0 29.7	92.5 32.2	128.0 46.9
37° C.	7	0.1	<i>P. vulgaris</i>	Average Actual	45.8 20.0	75.5 36.0	53.0 30.2	53.0 30.2	64.0 65.9	31.8 24.0	58.9 50.7
37° C.	8	0.1	<i>N. glutinosa</i>	Average Actual	67.6 34.7	53.5 61.5	105.0 30.5	105.0 30.5	100.0 32.6	94.7 15.7	121.0 30.6

<sup>a</sup> Numbers represent the average number of lesions per half-leaf obtained on 7 or more half-leaves of *Nicotiana glutinosa* or 16 or more half-leaves of *Phaseolus vulgaris* on inoculation with the designated virus-plus-pepsin preparation divided by the average number of lesions per half-leaf obtained on the other halves of the same leaves with the corresponding control preparation, multiplied by 100.

<sup>b</sup> Numbers represent the average of the actual number of lesions per half-leaf obtained on 7 or more half-leaves of *N. glutinosa* or 16 or more half-leaves of *P. vulgaris* on inoculation with the designated virus-plus-pepsin preparation.

ceeding period of digestion. When the hydrogen-ion concentration of the digestion mixtures was dropped to pH 3, the preparations containing 0.1 mg. of crystalline pepsin nitrogen per cc., with either purified virus or diluted infectious juice, produced no lesions on inoculation to *N. glutinosa* after 14 days' incubation at 37° C. The preparations of purified virus with 1 mg. of commercial pepsin per cc. required more than 14 days' incubation before loss of virus infectivity occurred. One preparation of diluted untreated juice plus commercial pepsin failed to produce lesions on inoculation to *N. glutinosa* after 14 days' incubation, while the other similar preparation still gave a few lesions after 28 days' incubation. When the amount of either crystalline or commercial pepsin was increased 10 times, the length of incubation necessary for a preparation that produced no lesions on inoculation to *N. glutinosa* was shortened markedly, in one case to less than 4 days, and in 3 other cases to less than 7 days. When the amount of either of the pepsin preparations was decreased to 1/10, 3 out of the 4 preparations produced a few lesions on inoculation to *N. glutinosa*, even after 97 days' incubation at 37° C. Furthermore, it may be seen that when the temperature of incubation was dropped from 37° C. to -15° C., all of the pepsin-treated virus preparations produced about as many lesions on inoculation to *N. glutinosa* as did the corresponding control preparations, even after a digestion period of 97 days.

A graphic representation of the results is given in figures 1 and 2. The results obtained with commercial pepsin and crystalline pepsin with both purified virus and infectious juice at the end of the various periods of digestion were averaged for each hydrogen-ion concentration and for each pepsin concentration and then plotted on a logarithmic scale against the time of digestion in days. In figure 1, the average of the number of lesions obtained with the 8 digestion mixtures listed in tables 1 to 4 containing 1.0 mg. commercial pepsin or 0.1 mg. crystalline pepsin nitrogen per cc. at pH 3 and 37° C., expressed as a percentage of the average number of lesions obtained with the controls, is plotted against the time of digestion. The results obtained with the preparations, of which there were 4 each at pH 4, 5, 6, 7 and 8, and containing comparable amounts of pepsin, were similarly treated and are also given in figure 1. It may be seen that the lines showing the results obtained at pH 5, 6, 7 and 8 fall very close to the line for the controls. This indicates that in each case the digestion mixtures produced about as many lesions as the corresponding control at the end of each period of digestion. The line for the results obtained at pH 4 gradually falls away from the control line, indicating a decreasing number of lesions with time of digestion. The line for the results obtained at pH 3 falls away sharply, thus indicating a sharp decrease in the average number of lesions at this hydrogen-ion concentration. Figure 2 pictures the results

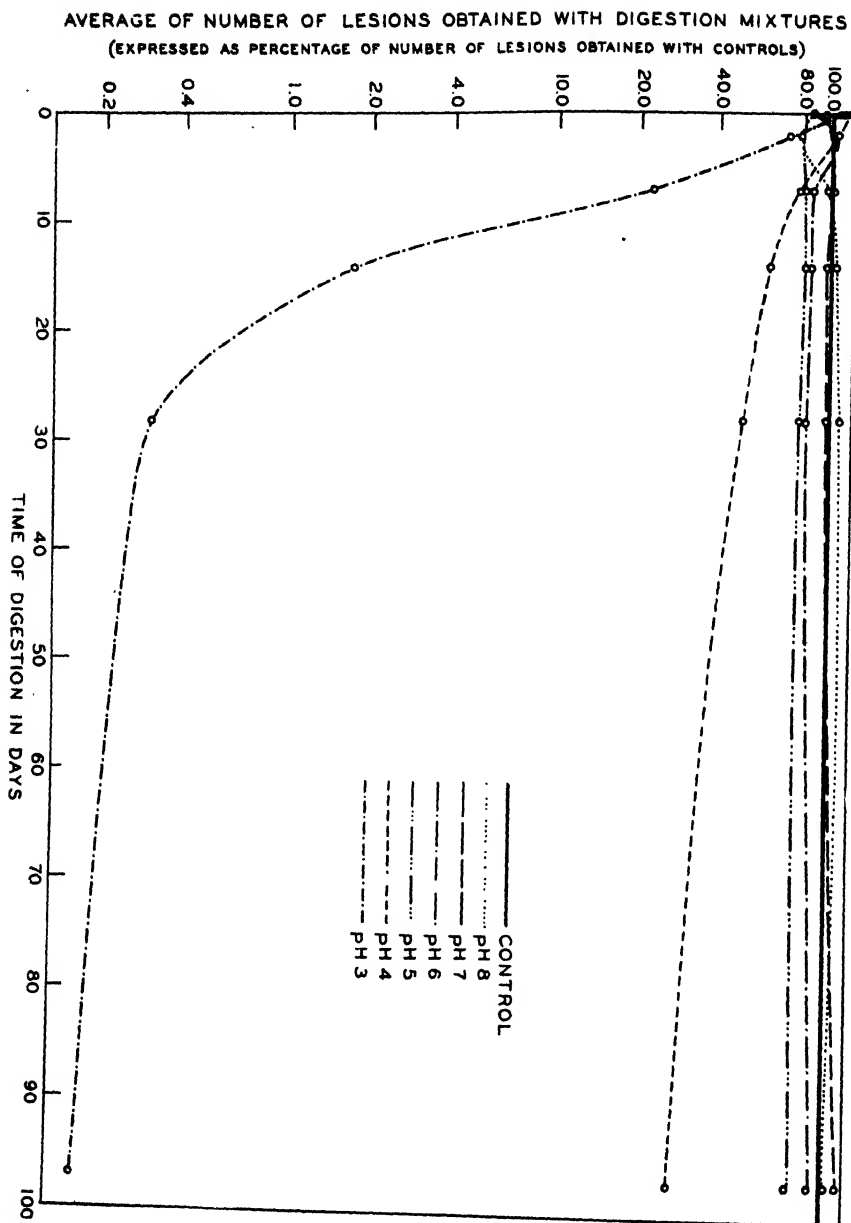


FIG. 1. Graph showing the effect of different hydrogen-ion concentrations on the inactivation of tobacco-mosaic virus with pepsin at 37° C. The lines showing the average results obtained at pH 5, 6, 7 and 8 fall very close to the line for the controls, thus indicating little or no inactivation of virus. The line showing the results obtained at pH 3 drops sharply, thus indicating a rapid inactivation of virus.

obtained at pH 3 with 3 different pepsin concentrations and the result when the digestion mixture is maintained at  $-15^{\circ}$  C. instead of  $37^{\circ}$  C. The line indicating the results obtained with the mixtures containing the greatest pepsin concentration drops off almost at right angles to the control line, whereas the line depicting the results when only 1/100 as much pepsin is present falls off gradually. It also may be seen from figure 2 that the results obtained with an intermediate pepsin concentration at  $37^{\circ}$  C. give a line that falls off sharply, whereas the results obtained with portions of the same preparations, maintained at  $-15^{\circ}$  C., give a line that falls very close to that of the control.

The results show that pepsin has no appreciable effect on the infectivity of tobacco-mosaic virus at pH 7 or pH 8, and but slight effect at pH 5 or pH 6, even after prolonged incubation at  $37^{\circ}$  C. However, at pH 4, pepsin has a noticeable effect in reducing the infectivity of virus after prolonged incubation, and at pH 3, pepsin reduces the infectivity of the virus quite rapidly at  $37^{\circ}$  C. Small amounts of pepsin cause the reduction in infectivity to proceed slowly and, conversely, large amounts of pepsin cause the reaction to proceed rapidly. Amounts of pepsin, which at pH 3 and  $37^{\circ}$  C. would cause a complete loss of infectivity in a given period, fail to affect the infectivity when the preparations are kept the same length of time at  $-15^{\circ}$  C. Crystalline pepsin appears to be slightly more effective than commercial pepsin. This is due, probably, to the fact that, although the actual weight of commercial pepsin used in comparable experiments was 1.6 times that of crystalline pepsin, the latter is about 5 times more active (7) proteolytically than commercial pepsin. The virus in the purified preparations appears to be affected slightly more by pepsin than that in the diluted, untreated infectious juice. This may be due to the presence of antienzymes or the great amount of extraneous protein matter in the untreated juice.

Although the averaged numbers given in tables 1 to 4 serve to give an idea of the course of the reaction, a better approximation of the percentage of virus remaining in a given virus-pepsin digestion mixture could be obtained by a comparison of the number of lesions produced by the digestion mixture, with the number of lesions produced by dilutions of the control or untreated virus preparation. For example, if, on inoculation, a given digestion mixture produces about the same number of lesions as does the control virus preparation diluted 1 to 10, it should be safe to assume that about 10 per cent of the virus is left. If the number of lesions produced is about the same as that given by the control diluted 1 to 100, then probably only about one per cent of the virus remains. The estimation of the amount of virus left after various digestion periods is useful in arriving at the rate of the reaction.

Since most of the experiments were made at pH 3, portions of the original control preparations at pH 3 used in the tests listed in tables 1 to 4 were diluted 1 to 10 and 1 to 100 and tested on *Nicotiana glutinosa*. The purified virus was diluted with 0.1 M phosphate at pH 3, and the infectious juice was diluted with 0.001 N HCl in order to give solutions at pH 3. There is some loss of virus on standing at pH 3 and 37° C.; so after standing for 14 days, additional portions of the control preparations were similarly diluted and tested. This makes possible a differentiation between loss of virus due to standing at pH 3 and 37° C., and loss of virus due to the action

TABLE 5.—*Dilution of control preparations of purified virus<sup>a</sup> and of infectious juice<sup>b</sup>*

Dilution	Purified virus		Infectious juice	
	At once	After 14 days at 37° C.	At once	After 14 days at 37° C.
1	39.8 <sup>c</sup>	6.8	32.2	25.2
1-10	8.4	1.7	5.4	4.9
1-100	1.5	0.2	0.8	0.6

<sup>a</sup> Dilutions of purified virus were made with 0.1 M phosphate at pH 3.

<sup>b</sup> Dilutions of infectious juice were made with 0.001 N hydrochloric acid (pH 3).

<sup>c</sup> Numbers represent the average number of lesions per half-leaf on 7 or more leaves of *N. glutinosa*.

of pepsin. From the results of the tests, (Table 5) the percentage of virus remaining in the various digestion mixtures at pH 3, listed in tables 1 to 4, may be estimated. It may be seen that digestion mixtures of purified virus, producing about 8 lesions per half-leaf within a few days after starting the digestion, have lost about 90 per cent of the virus because of the action of pepsin, and mixtures giving about 1.5 lesions have lost about 99 per cent of the virus. Similarly, mixtures that have stood for about 14 days and that produce about 1.7 lesions have lost about 90 per cent of the virus, and mixtures that produce about 0.2 lesions have lost about 99 per cent of the virus. In the case infectious juice, digestion mixtures that produce about 5 lesions on inoculation after standing for any period up to 14 days have lost about 90 per cent of the virus, and when they produce about 0.7 lesions about 99 per cent of the virus has disappeared. It is realized that some variation may be expected, because the comparisons described above are between the numbers of lesions produced on different sets of plants inoculated at different times. However, in general, the method is useful.



It may be seen, for example, that 0.1 mg. crystalline pepsin nitrogen or 1 mg. commercial pepsin per cc. with purified virus inactivated about 90 per cent of the virus in 7 days at pH 3 and 37° C. Crystalline pepsin caused 100 per cent inactivation in 14 days, and commercial pepsin about 99 per cent inactivation in 14 days and 100 per cent inactivation in less than 28 days. When the concentration of either form of pepsin was increased 10 times, 90 per cent inactivation occurred in about 2 days, 99 per cent inactivation in about 4 days, and 100 per cent inactivation in less than 7 days. When the concentration of either form of pepsin was decreased 1/10, 90 per cent inactivation of virus did not take place until after 7 days, 99 per cent inactivation until about 28 days, and in the case of commercial pepsin 100 per cent inactivation in less than 97 days. When infectious juice was used at pH 3 with 0.1 mg. crystalline pepsin nitrogen or 1.0 mg. commercial pepsin per cc., it required longer than 14 days to obtain 90 per cent inactivation, and in 3 instances less than 14 days for 100 per cent inactivation. When the pepsin concentration was increased 10 times, less than 90 per cent inactivation occurred in 2 days, and 99 per cent in one case, and 100 per cent in the other case at the end of 4 days. When the pepsin concentration was decreased 1/10 about 90 per cent inactivation occurred after 14 days' digestion and 99 per cent after 97 days' digestion. It may be seen that, in general, the rate of inactivation is directly proportionally to the amount of pepsin present.

A consideration of the results given above in connection with the characteristics of pepsin (7, 3) as a proteolytic enzyme is desirable. Pepsin is inactive as a proteolytic agent at pH 7 or pH 8. It possesses practically no activity at pH 5 and pH 6, and only about 10 per cent of its maximum activity at pH 4. It has more than 50 per cent of its maximum activity at pH 3 and has its maximum activity at about pH 2. At the concentration used it is practically inactive at low temperatures such as -15° C., and, like most enzymes, is more active at a higher temperature, such as 37° C. Its activity varies directly with its concentration. Thus, from the results obtained in the experiments described above, and the facts known about the proteolytic activity of pepsin, it becomes obvious that pepsin affects the infectivity of the virus of tobacco mosaic only under the conditions which are favorable for peptic activity. Furthermore, the results indicate that the loss of virus infectivity occurs at a more or less definite rate, inasmuch as the inactivation was found to vary directly with the amount of pepsin present, the activity of the pepsin, and the length of the period of digestion. The results are, therefore, a definite indication that the loss of virus infectivity on peptic digestion is due to the proteolytic action of pepsin. This suggests that the virus of tobacco mosaic is a protein, which is susceptible to hydrolysis in the presence of pepsin.

There remained the possibility, however, that virus is not attacked by pepsin, but that split products of other proteins, present in the digestion mixture, might so affect either the virus, or the plant used to measure its infectivity, as to cause the virus to fail to manifest its infectivity on inoculation. This did not appear very probable, however, since if such were the case, one would expect a greater effect with the untreated infectious juice, which contains much more protein than does the purified virus preparation. As may be seen by a comparison of the results given in table 1 and 3 with those given in tables 2 and 4, such was not the case,

TABLE 6.—*The addition of purified virus to pepsin-inactivated virus preparations*<sup>a</sup>

	Tested →	At once	After 2 days	After 4 days	After 13 days	After 23 days
1 volume commercial pepsin—purified virus mixture after 7 days' incubation (listed top table 1)	Average <sup>b</sup>	90.1	57.7	44.5	9.0	9.0
+ 1 volume purified virus	Actual <sup>c</sup>	70.8	25.2	17.1	1.9	3.0
1 volume commercial pepsin—infectious juice mixture after 7 days' incubation (listed top table 2)	Average	88.0	50.9	41.0	28.0	9.1
+ 1 volume purified virus	Actual	111.2	34.8	13.1	4.3	4.2
1 volume crystalline pepsin—purified virus mixture after 7 days' incubation (listed top table 3)	Average	94.0	73.8	43.5	19.8	12.0
+ 1 volume purified virus	Actual	97.4	31.6	15.4	4.1	3.2
1 volume crystalline pepsin—infectious juice mixture after 7 days' incubation (listed top table 4)	Average	109.5	52.2	51.5	37.0	18.9
+ 1 volume purified virus	Actual	80.0	34.8	26.4	9.4	9.3

<sup>a</sup> All preparations were at pH 3 and incubated at 37° C.

<sup>b</sup> Numbers represent the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* on inoculation with the designated virus-pepsin preparation divided by the average number of lesions per half-leaf obtained on the other halves of the same leaves with the corresponding control preparation, multiplied by 100.

<sup>c</sup> Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* on inoculation with the designated virus-pepsin preparation.

since the untreated infectious juice was affected less than the purified virus preparation. Furthermore, when purified virus was added to pepsin-virus preparations, which had been subjected to incubation at 37° C. and pH 3 until no lesions appeared on inoculation to *Nicotiana glutinosa*, the infectivity of the added virus was not changed appreciably. The results of such an experiment are given in table 6. Portions of the same 4 pepsin-virus preparations listed at the top of tables 1, 2, 3, and 4, containing 1.0 mg. of crystalline pepsin nitrogen per cc. or 10 mg. of commercial pepsin per cc. with both diluted purified virus and diluted untreated infectious juice, were used after 7 days' incubation at 37° C. and pH 3. One volume of each of the 4 preparations described above was added to an equal volume of a purified virus preparation and the resulting mixtures were immediately tested for virus infectivity by the half-leaf method on *N. glutinosa* against controls. Controls for the 2 pepsin-purified virus preparations consisted of the purified virus preparation plus an equal volume of 0.1 M phosphate at pH 3. Controls for the 2 pepsin-infectious juice preparations consisted of the purified virus preparation plus an equal volume of diluted healthy Turkish tobacco juice at pH 3. The tests for virus infectivity were repeated after incubation at 37° C. for 2, 4, 13, and 23 days. It may be seen from table 6 that the 4 pepsin-treated virus preparations, upon addition of new virus, gave 90.1, 88.0, 94.0, and 109.5 per cent, respectively, as many lesions on *N. glutinosa* as did the corresponding control preparations. The average of the results obtained with the 4 preparations is shown in figure 2 as a continuation of the line giving the results with the highest ( $C \times 10$ ) concentration of pepsin. The results demonstrate that split products in pepsin-virus digestion mixtures have no appreciable effect on either virus added to such mixtures or the plant used to measure the infectivity of the virus. Furthermore, the results given in table 6 and portrayed in figure 2 show that virus, added to a pepsin-virus digestion mixture, is susceptible to digestion, since the infectivity of the added virus decreased on incubation.

Many attempts have been made to regain the infectivity of virus that has disappeared as a result of digestion with pepsin. Heating for 20 minutes at 70° C., or diluting with water, 0.1 M phosphate at pH 7, or healthy Turkish tobacco juice, failed to restore the infectivity of virus inactivated with pepsin, although such procedures increase the infectivity of virus in trypsin-virus mixtures. The addition of infectious juice or purified virus, inactivated by heat or by either high or low hydrogen-ion concentrations, and the addition of activated charcoal to pepsin-inactivated virus failed to restore the infectivity of the virus. Changing the hydrogen-ion concentration of virus preparations inactivated by means of pepsin to pH 5 or pH 7 also failed to cause the infectivity of the virus to return, even

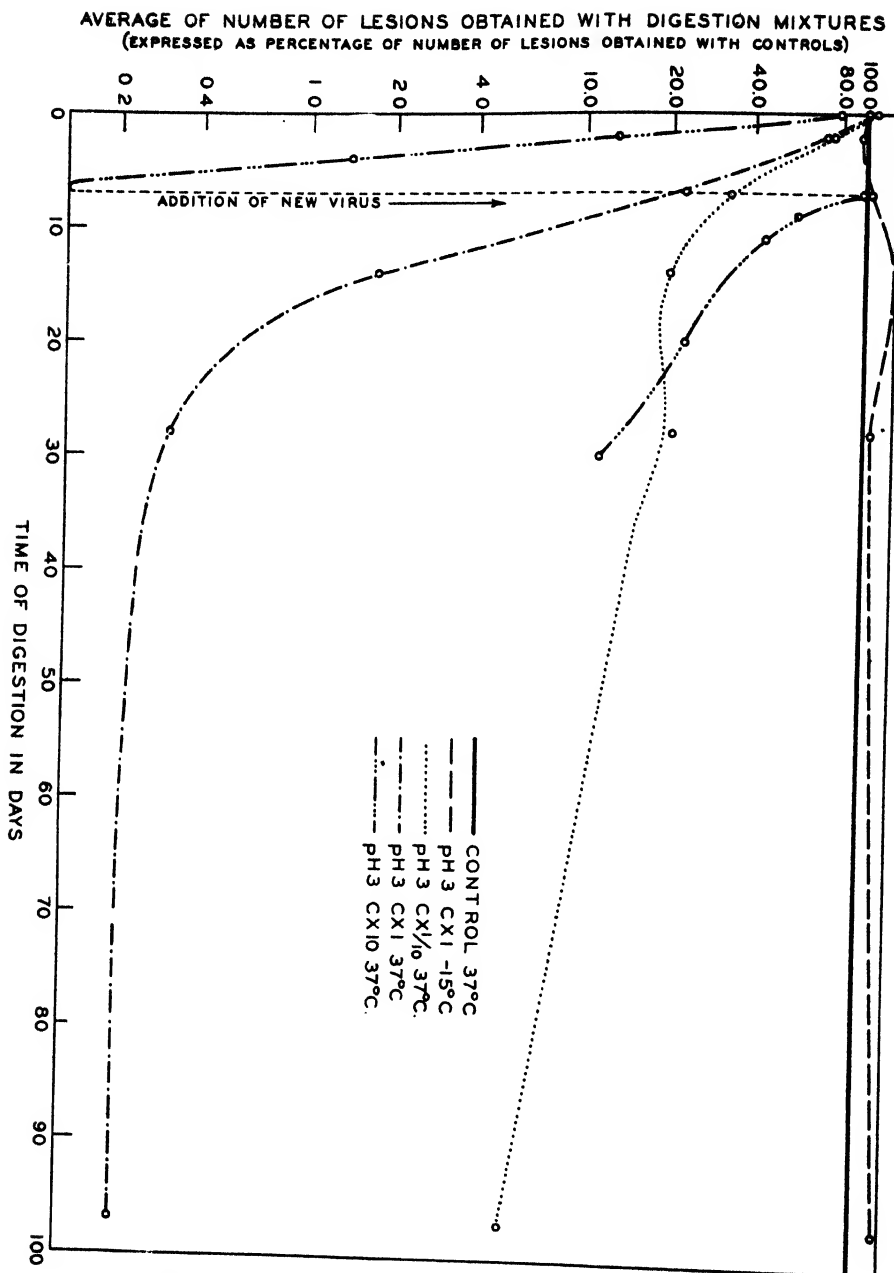


FIG. 2. Graph showing the effect of different pepsin concentrations and of a different digestion temperature on the inactivation of tobacco-mosaic virus with pepsin. The line showing the average results obtained with the pepsin concentration ( $C \times 1$ )

after long standing. It seems difficult to avoid the conclusion that tobacco-mosaic virus is a protein, or closely associated with a protein, which may be hydrolyzed with pepsin.

#### The Action of Pepsin on Different Strains of Tobacco-mosaic Virus

An experiment was made for the purpose of determining whether digestion with pepsin would inactivate other strains of tobacco-mosaic virus. The strains used in this experiment were aucuba mosaic, a masked strain (4) and a yellow strain (5) of tobacco-mosaic virus previously described (8). The aucuba mosaic was a purified sample prepared from infectious tomato juice. The masked and yellow strains were in juice from infected Turkish tobacco plants. Samples of each of the 3 viruses were taken to pH 3.5 by the addition of 0.2 N HCl with vigorous stirring, and each sample was then divided into 2 equal portions. To one portion of a sample of each virus was added an equal volume of 0.001 N HCl containing 2 mg. of crystalline pepsin nitrogen per cc., and to the other portion was added an equal volume of 0.001 N HCl. The hydrogen-ion concentrations of the 3 pepsin-treated preparations and of the 3 controls were found to be  $\text{pH } 3.5 \pm 0.2$ . Immediately after the addition of pepsin, the 3 preparations were tested for virus infectivity on leaf-halves of *Nicotiana glutinosa* against the 3 controls, and all preparations were then placed in a room at 37° C. They were removed and again tested after 1, 2, 3, 5, and 8 days. The results of the tests are given in table 7. It may be seen that all 3 strains of tobacco-mosaic virus are inactivated on digestion with pepsin. Dilutions of the controls indicate that less than 1 per cent of the virus remains in the virus-pepsin digests after 8 days' incubation at 37° C. The complete inactivation of the masked strain after 8 days' incubation is due, probably, to the fact that its concentration in the original preparation was much less than the concentrations of the other 2 viruses. The results indicate that the action of pepsin on strains of tobacco mosaic is similar to the action on tobacco-mosaic virus itself.

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used in most of the experiments at 37° C., drops away from the line for the controls sharply, thus indicating a rapid inactivation of virus. The line for the results obtained when the pepsin concentration is reduced to 1/10 ( $C \times 1/10$ ) falls off very gradually, whereas the line for the results obtained when the pepsin concentration is increased 10 times ( $C \times 14$ ) falls off almost at right angles. When new or active virus was added to the latter solutions ( $C \times 10$ ) there was but little immediate effect on the infectivity of the added active virus. However on digestion the activity of the added virus decreased as indicated by the extension of the line for the results obtained with pepsin concentration  $C \times 10$ . The line for the results obtained with the usual pepsin concentration ( $C \times 1$ ) at -15° C. falls very close to the line for the controls, thus indicating little or no inactivation of virus.

TABLE 7.—*The action of pepsin on different strains of tobacco-mosaic virus*

	Tested →	At once	After 1 day	After 2 days	After 3 days	After 5 days	After 8 days
Purified aucuba mosaic	Average	100.0 <sup>a</sup>	123.0	64.5	41.8	16.1	2.6
+ 1.0 mg. pepsin N per cc. (pH 3.5 ± 0.2)	Actual	165.0 <sup>b</sup>	89.0	56.0	64.0	16.0	2.0
Infectious juice masked strain of virus	Average	76.5	99.0	56.5	45.3	10.0	0.0
+ 1.0 mg. pepsin N per cc. (pH 3.5 ± 0.2)	Actual	144.0	128.0	68.0	57.0	8.0	0.0
Infectious juice yellow strain of virus	Average	81.2	94.5	53.5	50.8	37.7	8.1
+ 1.0 mg. pepsin N per cc. (pH 3.5 ± 0.2)	Actual	289.0	489.0	88.0	281.0	63.0	19.0

<sup>a</sup> Numbers represent the average number of lesions per half-leaf obtained on 10 or more half-leaves of *Nicotiana glutinosa* with the designated virus-plus-pepsin preparation divided by the average number of lesions per half-leaf obtained on the other halves of the same leaves with the corresponding control preparation, multiplied by 100.

<sup>b</sup> Numbers represent the average of the actual number of lesions per half-leaf obtained on 10 or more half-leaves of *N. glutinosa* on inoculation with the designated virus-plus-pepsin preparation.

#### DISCUSSION

The experiments described in this report show that the infectivity of the virus of tobacco mosaic is lost on digestion with pepsin only under conditions favorable for proteolytic activity. There is no appreciable immediate change in the infectivity of the virus on addition of pepsin as measured on *Nicotiana glutinosa* regardless of the hydrogen-ion concentration of the virus-pepsin mixture. This is in striking contrast to the effect caused by trypsin where the addition of even very small amounts of trypsin caused an immediate loss of virus infectivity. Pepsin has no effect on the infectivity of the virus either at once or on standing at pH 7 or pH 8, hydrogen-ion concentrations at which pepsin is inactive as a proteolytic agent. Pepsin possesses practically no proteolytic activity at pH 5 or pH 6, and, likewise, has almost no effect on the infectivity of virus at pH 5 or pH 6. At pH 4 pepsin manifests about 10 per cent of its maximum proteolytic activity and at this hydrogen-ion concentration slowly, without appreciable immediate effect, inactivates the virus. Pepsin has its maximum proteolytic

activity at about pH 2, but it is impossible to carry out experiments at this hydrogen-ion concentration, since the virus is inactivated at pH 2. However, pepsin exhibits over 50 per cent of its maximum proteolytic activity at pH 3, a hydrogen-ion concentration that inactivates virus very slowly and it was found that at pH 3 preparations of both commercial and crystalline pepsin inactivate purified virus and virus in infectious juice very rapidly. Similar results were obtained with aucuba mosaic, and a yellow and a masked strain of tobacco-mosaic virus. The rate of the inactivation was found to be proportional to the amount of pepsin present. No inactivation occurs if the digestion mixture at pH 3 is kept at  $-15^{\circ}$  C., at which temperature pepsin is practically inactive. The fact that pepsin inactivates virus only under conditions that are favorable for proteolytic activity, and that the rate of inactivation varies directly with the pepsin concentration, is evidence that the loss of virus infectivity is due to the proteolytic action of pepsin.

Pepsin-virus digestion mixtures, in which the virus has been inactivated, have no appreciable effect on the infectivity of virus added to such mixtures. This fact indicates that such digestion mixtures are not toxic to virus or to plants susceptible to virus, and, hence, that any active virus present in such mixtures would manifest its infectivity on inoculation to susceptible plants. The failure of pepsin-virus mixtures to produce lesions is, therefore, an indication that no active virus is present.

The infectivity of pepsin-inactivated virus was not regained by heating or by diluting. This fact indicates that the inactivation is not similar to the loss of infectivity caused by the addition of trypsin. The fact that the addition of juice from healthy Turkish tobacco plants or of infectious juice that has been inactivated by heat or by high or low hydrogen-ion concentrations does not reactivate the virus in pepsin-virus digestion mixtures, is an indication that the inactivation is not due to the loss of substances or hydrolysis of proteins present in the above-described preparations. The results indicate, therefore, that the inactivation of tobacco-mosaic virus on digestion with pepsin is due to the proteolytic action of pepsin. Because of the specificity of pepsin, this suggests that the virus is a protein, or closely associated with a protein, that may be hydrolyzed with pepsin.

It is believed that Lojkin and Vinson (6) did not report inactivation of virus on digestion with pepsin because, as they themselves suggest, virus preparations at hydrogen-ion concentrations at which pepsin possesses little or no proteolytic activity were used. Most of their experiments appear to have been carried out at pH 4.7 or above, hydrogen-ion concentrations at which pepsin is almost inactive. One experiment was performed at pH 3.3, but they reported the control was also inactivated. This result

may have been due, not to inactivation of the control, but to the failure of the Turkish tobacco plants, which they used as test plants, to respond to virus at pH 3.3. It has been found that the susceptibility of Turkish tobacco plants to virus is markedly lowered at pH 3, while that of *Nicotiana glutinosa* is but slightly affected. In one experiment with pepsin at pH 3.5, using *N. glutinosa* as the test plant, Lojkin and Vinson found that the pepsin digest gave 89.5 per cent as many lesions as did the control after 2 days' incubation, and only 50.5 per cent as many lesions as did the control after 11 days' incubation. Although they came to the conclusion that pepsin does not attack the virus, it is believed that the decrease in the number of lesions mentioned above in their experiment at pH 3.5 was due to the action of pepsin on the virus.

Caldwell (1) stated that in his experiments he prepared an inoculum from tomato leaf tissue and diluted this 1 to 50. He did not give the hydrogen-ion concentration of his preparation, but if he made the dilution with water and did not otherwise change the hydrogen-ion concentration, the preparation would be near pH 5.8. Since pepsin is practically inactive above pH 4, peptic digestion would not be expected.

#### SUMMARY

Pepsin has no appreciable immediate effect on the infectivity of tobacco-mosaic virus or its strains including aucuba mosaic, a yellow and a masked strain at pH 3 to pH 8, inclusive, as measured on *Nicotiana glutinosa*.

Pepsin has no appreciable effect on the infectivity of tobacco-mosaic virus at pH 7 and pH 8 and but little effect at pH 5 and pH 6, even on prolonged digestion at 37° C. Pepsin slowly inactivates this virus at pH 4 on prolonged digestion at 37° C.

Pepsin inactivates tobacco-mosaic virus, and the strains mentioned above, rapidly at pH 3 and 37° C. Pepsin fails to inactivate virus at pH 3 and a temperature of -15° C., even on prolonged standing.

The rate of inactivation of virus was found to be proportional to the concentration and activity of pepsin, and to the time of digestion.

Pepsin-virus digestion mixtures, containing inactive virus, have no appreciable immediate effect on the infectivity of virus added to such mixtures. This indicates that materials toxic to virus or test plant are not produced on digestion of virus with pepsin.

Attempts to regain the infectivity of virus inactivated by pepsin have resulted in failure.

Since pepsin inactivates virus only under conditions favorable for proteolytic activity, and since the rate of inactivation of virus varies directly with the concentration of active pepsin, it is concluded that the



inactivation of virus is due to the proteolytic action of pepsin. This suggests that the virus of tobacco mosaic is a protein, or very closely associated with a protein, which may be hydrolyzed with pepsin.

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# SCLEROTIAL ROT OF CORN CAUSED BY RHIZOCTONIA ZEAE, N. SP.

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In the summer of 1932 several rotted mature ears of corn, *Zea mays* L., were found at Quincy, Florida, that were somewhat similar in appearance to ears rotted either by *Diplodia zeae* (Schw.) Lev. or *D. macrospora* Earle. Certain characteristics, however, indicate that the rot was not caused by either of these fungi. These diseased ears had a somewhat sour fungoid odor, which is different from the dry, musty odor accompanying ears rotted either by *D. zeae* or *D. macrospora*. The outer husks of some of the diseased ears were covered with small sclerotia that are not found on ears rotted by either of the above fungi. Isolations of the fungus were made from these ears and the pathogenicity of the fungus to corn seedlings in the greenhouse and laboratory and to ears of corn in different stages of development in the field has been determined. Studies on the morphology and physiology of the fungus showed that the fungus belonged in the genus *Rhizoctonia*. Results of these investigations and a description of the disease and the parasite are given herewith.

## SYMPTOMS

Diseased ears are shrunk and often tightly clasped by the husks because of the mycelium of the fungus. In the early stages of infection diseased ears and their husks are covered with the salmon pink mycelium, and in the later stages they have a dull grayish appearance due to the change in color of the mycelium. The outer husks often are covered with small, immature white, as well as mature brown to dark brown, sclerotia attached to the mycelial strands. The mycelium is visible on the kernels and between the kernel rows (Fig. 1, B), as with ears rotted by *Diplodia zeae* or *D. macrospora*, but it may or may not be visible on the kernels of ears that are not severely diseased. On some diseased ears the mycelium can be seen only on the base of the kernels.

## THE CAUSAL ORGANISM

### Isolations

The first isolations were made from kernels of the diseased ears (Fig. 1, B) originally discovered. Individual kernels were taken from the ears and were surface-sterilized in corrosive sublimate 1:1000 for 10 minutes. They were planted in Petri dishes containing potato-dextrose agar (Fig.

2, B). The mycelium grew from the kernels and had covered the entire surface of the medium within 3 days. After 7 days numerous small sclero-

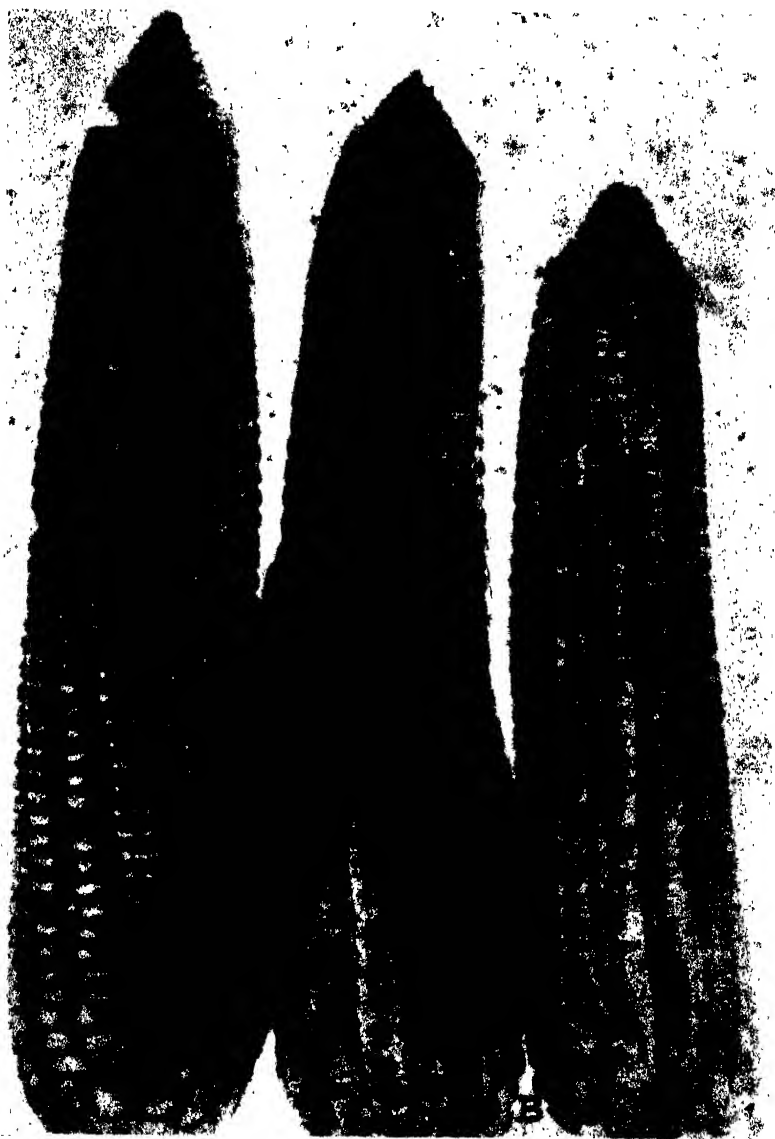


FIG. 1. A. Healthy ear. B. Two ears rotted from natural infection by *Rhizoctonia seae*.

tia, characteristic of the genus *Rhizoctonia*, were produced. A large number of isolations were made from such diseased ears and the same fungus was obtained consistently.



FIG. 2. A. Ear of corn rotted by artificial inoculation of *Rhizoctonia zeae*. Note numerous sclerotia on outer husks. B. Pure cultures of *R. zeae* growing from diseased surface-sterilized kernels on potato-dextrose agar.

## Pathogenicity

*Seedlings.*—Healthy corn seedlings, growing in the greenhouse and in the field, were inoculated with sclerotia and mycelium of the fungus growing on sterilized corn meal. The seedlings were inoculated by placing the

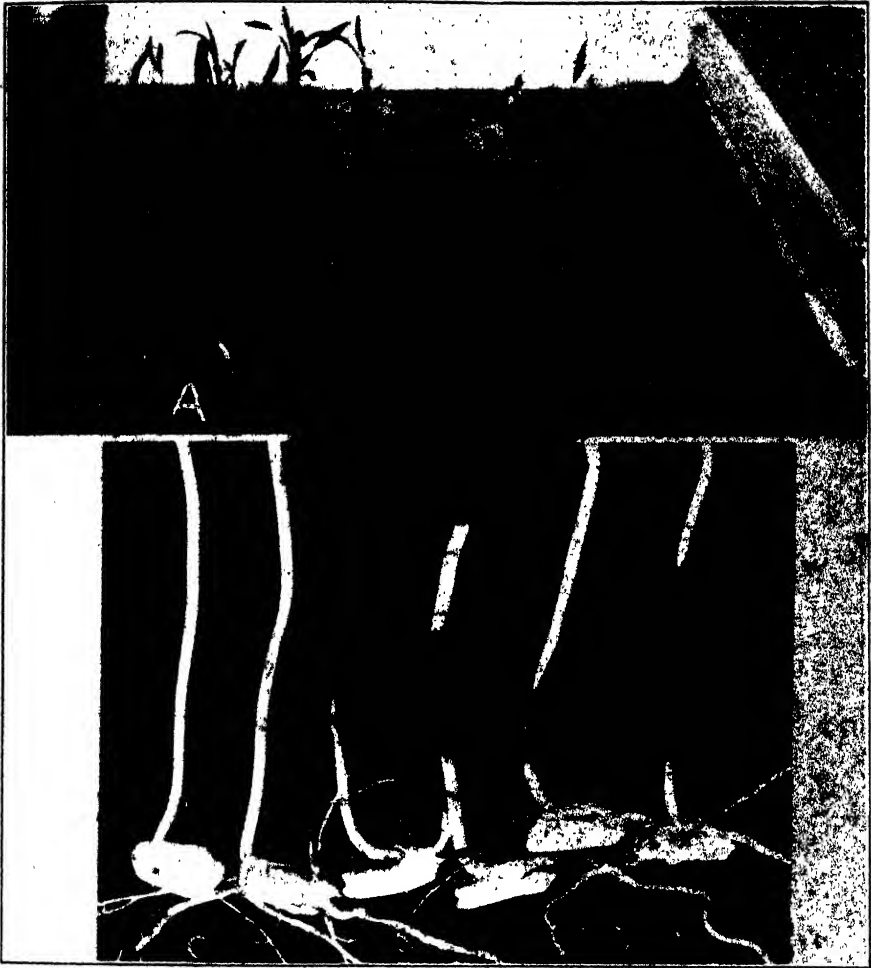


FIG. 3. A and B. Corn seedlings growing in greenhouse in sterilized soil. A. Non-inoculated (100 per cent emergence). B. Inoculated with *Rhizoctonia seae* (31 per cent emergence). C and D. Grown in laboratory in sterilized soil. C. Noninoculated. D. Inoculated with *R. seae*.

inoculum in the soil around the roots, on the surface of the soil around the stalks, and in incisions in the stalks. The fungus grew readily and produced numerous sclerotia on the surface of the soil and in the incisions in

the stalks, but only slight infection was produced on the nonwounded seedlings.

In another test healthy sterilized kernels were planted in sterilized soil on a greenhouse bench. This planting consisted of a plot of 8 rows, 10 hills per row and 1 kernel per hill. The soil in part of the plot was inoculated by placing a kernel with the fungus growing on it in each hill with the healthy kernel, the corn in the other part of the plot was the check. Only 31 per cent emergence was obtained in the inoculated soil, while 100



FIG. 4. Sclerotia. A. On surface of inoculated soil.  $\times 7$ . B. On husk.  $\times 6$ .

per cent emergence was obtained in the check (Fig. 3, A and B). The fungus either killed the kernels before they germinated or killed the seedlings before they emerged. It failed to kill any of the seedlings after they had emerged. The fungus grew readily in the soil and produced numerous sclerotia on the soil surface (Fig. 4, A).

A test was conducted in the laboratory to study the parasitism of the fungus to corn seedlings under controlled conditions. Twenty healthy sterilized corn kernels were evenly distributed on the bottom of each of 4

crystallization dishes. To 2 of the dishes was added sterilized soil with which inoculum, consisting of the fungus growing on sterilized corn meal, had been mixed. To the remaining 2 dishes, sterilized soil, mixed with sterilized corn meal, was added to serve as checks. Sufficient water was added to each dish to bring the soil up to a desirable moisture content. Each day the dishes were weighed and water was added to bring them to their original weight. The dishes were placed in electrically controlled chambers adjusted to a constant temperature of 30° C. After a period of 9 days, the seedlings were washed free from the soil and observed for infection. Sixty per cent of the seedlings in the inoculated soil were infected, while no infection developed in the checks. The infected seedlings were characterized by a rotting of the mesocotyl at or just below the soil surface (Fig. 3, D), while the roots were free from infection.

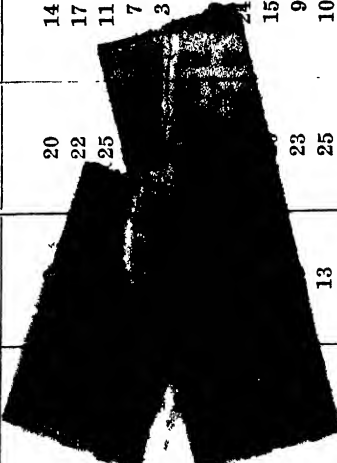


FIG. 5. Cross sections of sclerotia of *Rhizoctonia zeae* of various sizes and shapes in roots of corn seedlings.  $\times 30$ .

Healthy sterilized kernels also were planted on potato-dextrose agar slants in test tubes and placed in a chamber at a constant temperature of 30° C. After these kernels had germinated the seedlings were inoculated with sclerotia or a small portion of the mycelium. Infection of the seedlings was evident after 3 to 4 days and most of the seedlings were dead at the end of 8 days. The mycelium invaded the seedling roots and mesocotyls, and sclerotia were formed in these parts, as well as on the surface, of the host. Cross sections of some of the roots showing sclerotia of various shapes, and sizes as they are formed in the roots are shown in figure 5.

In the above tests the amount of inoculum probably was in excess of what normally occurs in the field, but the tests indicate the parasitic capabilities of the fungus on corn seedlings.

TABLE 1.—Comparative susceptibility of ears of two varieties of field corn in different stages of development of *Rhizoctonia zeae*

Date of inoculation	Method of inoculation	Stage of development of ears	Condition of inoculated ears at time of harvesting					
			Whatley			Cuban Yellow Flint		
			No. inoculated	No. with rotted kernels	No. with sclerotia on husks	No. inoculated	No. with rotted kernels	No. with sclerotia on husks
June 21 . . .	Inoculum inserted in ear tips	Blister	25			20	14	10
“ 30 . . .	“	Early Milk	25			22	17	13
July 6 . . .	“	Milk	24			25	11	6
“ 15 . . .	“	Late Milk	25				7	6
“ 22 . . .	“	Dent	24				3	4
June 21 . . .	Inoculum inserted in ear shanks	Blister	25					19
“ 30 . . .	“	Early Milk	25					23
July 6 . . .	“	Milk	25				15	15
“ 15 . . .	“	Late Milk	25			23	9	8
“ 22 . . .	“	Dent	21		13	25	10	10
June 21 . . .	Inoculum dropped in crevice between ear and sheath	Blister	24	4	6	20	3	4
“ 30 . . .	“	Early Milk	22	3	4	20	2	3
July 6 . . .	“	Milk	20	3	3	23	1	2
“ 15 . . .	“	Late Milk	24	2	4	25	1	1
“ 22 . . .	“	Dent	23	1	2	20	0	0



*Ears.*—Although the fungus is capable of inhabiting the soil and causing infection of corn seedlings in the soil, observations made thus far indicate that it causes most damage to the ears of the corn plant. Experiments were conducted in the field in which ears of corn of a white dent and a yellow flint varieties were inoculated at different stages of growth. The inoculum consisted of sterilized corn kernels on which the fungus was growing. At intervals of 6 to 9 days from June 21 to July 22, inclusive, 20 to 25 ears were inoculated by each of 3 methods, as follows: (1) Inoculum placed in wound in ear tip; (2) inoculum placed in wound in ear shank; (3) inoculum dropped in crevice between the ear and sheath.

The inoculated ears were harvested August 20 and examined for visible signs of infection. As shown in table 1, infection was obtained by all 3 methods of inoculation. The method producing the highest percentage of infected ears with visible signs of rotting was that in which the inoculum was inserted in the shank. By this method the inoculum was inserted in the moist shank of the ear where it was protected from drying. Ears inoculated by inserting the inoculum into the tips gave a much higher rate of infection than was obtained by dropping the inoculum between the ear and sheath. The inoculum dropped in crevices between the ear and sheath suffered a great deal more from lack of moisture than that inserted in the ear tips.

In these tests ears in the early stages of growth were more susceptible to infection than those in the later stages of development. This was probably because the young, tender ears were easily penetrated and contained more moisture to support the growth of the fungus than older ears that were beginning to dent and harden. The Whatley or white dent was more susceptible to infection than the Cuban yellow flint variety, and especially so as the ears approached maturity.

A majority of the infected ears were invaded and thoroughly rotted by the fungus (Fig. 6), and sclerotia developed on the husks of many of them. The mycelium apparently enters the kernel at its base, since the fungus is first detected in this region. From this point the mycelium grows throughout the kernel and is found in both the endosperm and embryo. The starch grains in the kernels are completely destroyed, thus reducing the weight of the ears. In addition to using up the food stored in the kernels, the fungus completely destroys the viability of the kernels.

#### Taxonomy

A study of the literature has failed to reveal reports of a similar fungus attacking corn and especially the ears. Although several species of *Rhizoctonia* have been reported on corn, all of them appear quite distinct from the species discussed herein. Mackie (1) reported *Sclerotium bataticola*

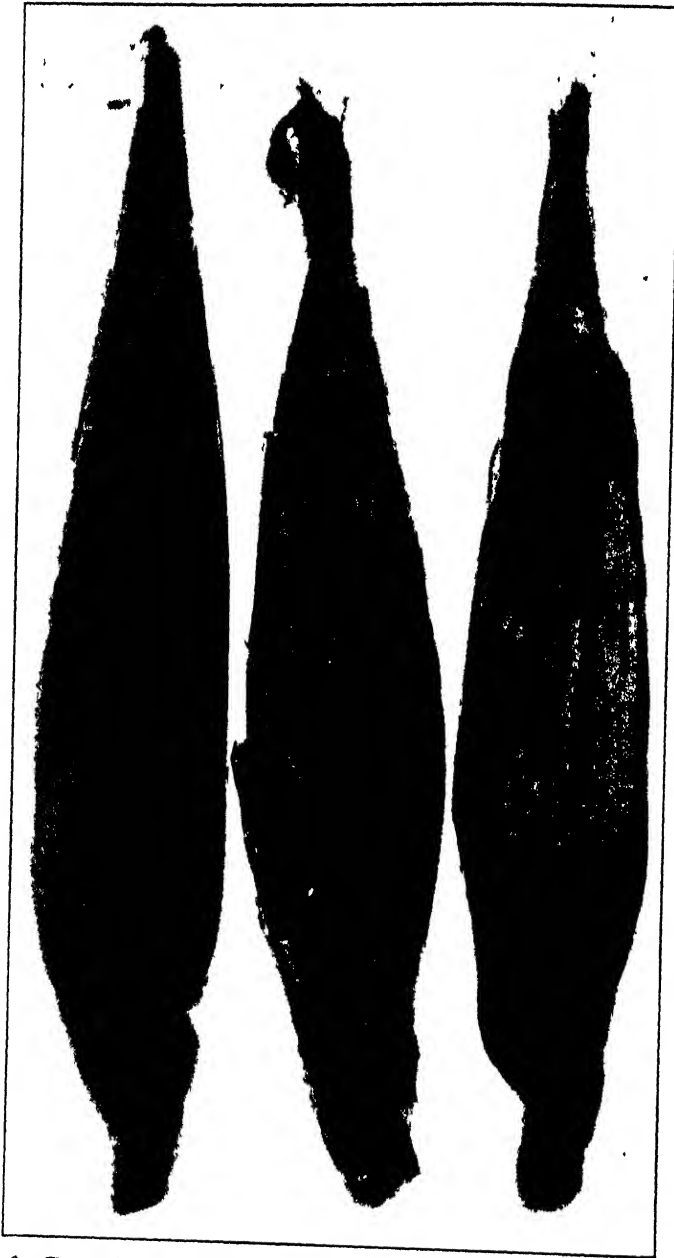


FIG. 6. Ears of corn inoculated in the tip with cultures of *Rhizoctonia zeae*. Healthy ear (left).

Taub. as causing a dry rot of maize, but this dry rot was limited to the pith of the stalk. He (2) later called it charcoal rot, as it was first named by Taubenhaus (6) under the above binomial. This form, bearing only sclerotia, is generally accepted to be *Rhizoctonia bataticola* (Taub.) Butler, as reported by Mackie (2). Peltier (4) reported *Rhizoctonia solani* Kühn on corn roots in the field. Rolfs (5), in 1905, reported *Corticium vagum* B. & C. on sweetcorn in Florida, but did not state on which part of the plant it was found. A somewhat similar *Rhizoctonia*, but one that produces smaller sclerotia than the one described herein, was reported by Matz (3) on fig in Florida.

Since the disease on corn is undescribed and is caused by a fungus different from any species of *Rhizoctonia* previously described, it is considered to be a new species. Consequently, the parasitic fungus is described as follows:

***Rhizoctonia zeae*, sp. nov.**—Sclerotia submerged and superficial in culture, 0.5 to 1.0 mm. in diameter; mostly superficial on host, 0.1 to 0.5 mm. in diameter, white when young, brown to dark brown at maturity; homogeneous in structure and color, hard at maturity, usually single, sometimes conglomerated, produced abundantly on host and in culture; hyphae 4 to 10 $\mu$  wide; at first, hyaline and granular, reddish-brown in old cultures, salmon-pink on host, later becoming dull gray, multiseptate, constricted at septum.

Produces ear rot of corn, *Zea mays*; sclerotia produced on outer ear husks. (No. 9414—Fla. Agr. Exp. Sta. Herb.).

### Morphology and Physiology

The general morphological characteristics of the above-described fungus indicate that it belongs to the genus *Rhizoctonia*. It has not yet been connected with a perfect stage. In young cultures the hyphae ramify profusely; the branches first appear to be more or less parallel to the main axis and constricted at the base, which is characteristic of the genus *Rhizoctonia*. Later, the hyphae branch irregularly, usually at right angles to the parent hyphae, and become multiseptate. The mycelium grows rapidly, covering the surface of media in a 9 cm. Petri dish within 3 days. After 7 days numerous sclerotia are embedded in the medium, and on the surface (Fig. 2, B). The origin and development of the sclerotia were studied and different stages are shown in figure 7. Although the formation of a sclerotium may begin from a single hypha, it appears to start usually from an aggregation of hyphae. By repeated branching and anastomosing of these hyphae, it soon becomes a dense mass with short hyphae protruding and eventually a mature sclerotium (Fig. 7, D). Cross

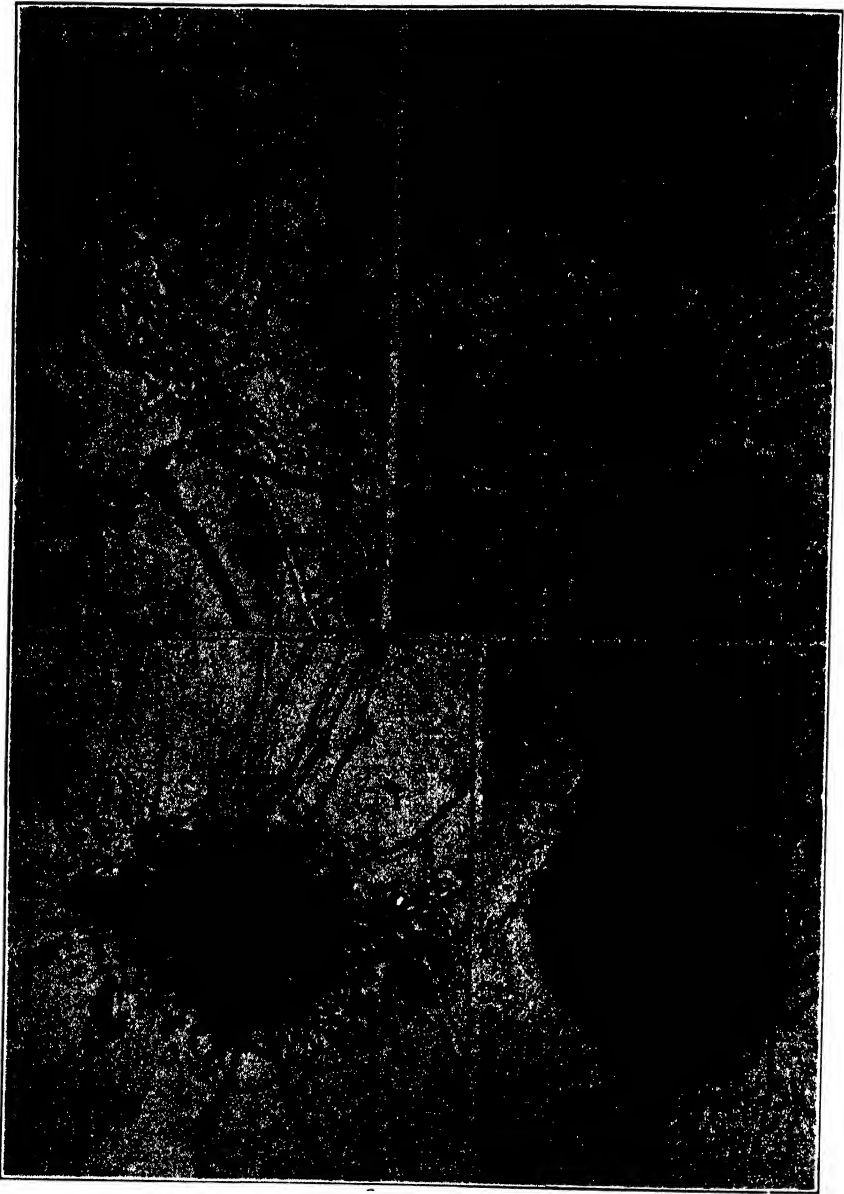


FIG. 7. Stages in the formation of a sclerotium of *Rhizoctonia zeae*. A. Small mass of entangled hyphae in the early stage.  $\times 225$ . B. Later stage in the same.  $\times 225$ . C. Later stage with branches protruding, some of which are rebranching.  $\times 175$ . D. The network rounded into a mature sclerotium, with sclerotia beginning to form on each side apparently on the same parent hyphae.  $\times 60$ .

sections of mature sclerotia (Fig. 5) show that they consist of interwoven hyphae, resulting in a homogeneous structure.

At the end of a year, pure cultures of the fungus were still viable. Sclerotia of the organism were transplanted for several generations and the original characters of growth, form, and size of sclerotia were maintained throughout the period.

*Relation of Temperature to Mycelial Growth in Culture.*—The culture of the organism used in these studies was isolated from corn ears and had been grown in pure culture on potato-dextrose agar. A series of 60 plates of potato-dextrose agar were inoculated in the center with uniform bits of mycelium from a 3-day-old culture, and 5 plates were incubated for 3 days at each of 12 different temperatures ranging from 11 to 42° C. At the end of 3 days the diameters of the colonies were measured and the average diameter of the 5 plates at each temperature is presented in table 2.

TABLE 2.—*The relation of temperature to the mycelial growth of Rhizoctonia zeae on potato-dextrose agar*

Temperature in degrees Centigrade	11	14	17	21	24	27	30	33	36	38	40	42
Average diameter of colonies in centimeters	0	.7	1.2	3.6	6.1	6.6	7.1	7.8	6.1	3.4	.3	0

The mycelium at the lower temperatures (14 to 21° C.) was extremely thin. Colonies of approximately the same diameters at higher temperatures were more dense. At temperatures approaching the optimum and at the optimum the mycelium was dense and whitish; later the cultures became dotted with sclerotia as shown in figure 2, B. In old cultures the mycelium becomes reddish-brown. The optimum temperature for growth in this test was close to 33° C., the minimum between 11° and 14° C., and the maximum between 40° and 42° C. The fact that the fungus grew at 38° and slightly at 40° C. will account for its ability to thrive and cause infection of corn ears that usually are exposed to the direct sunlight and high air temperatures prevalent during the summer. Sclerotia stored in an electric oven at a constant temperature of 50° C. were still viable after 60 days. This temperature is much higher than that usually occurring in the field, and shows that the fungus is thoroughly capable of tolerating the high summer temperatures.

*Relation of Hydrogen-ion Concentration to Mycelial Growth.*—*Rhizoctonia zeae* was grown on a 2 per cent potato-dextrose agar adjusted to 12 different hydrogen-ion concentrations. Five test tubes, each containing 15 cc. of this medium, were used for each concentration, and the concentra-

tions used ranged from pH 2.5 to 9.0. The adjustments were made by adding the required number of drops of HCl and NaOH of different normalities, the determinations being made with a Youden Hydrogen-ion Concentration Apparatus. After the medium was adjusted, it was poured into Petri dishes, inoculated in the center with uniform bits of mycelium, and exposed to a constant temperature of 33° C. At the end of 3 days the diameters of the colonies were measured, and the average diameter of the 5 plates at each concentration is presented in table 3.

TABLE 3.—*The relation of hydrogen-ion concentrations of potato-dextrose agar to the rate of mycelial growth of Rhizoctonia zeae at 33° C.*

pH reaction . . . . .	2.5	3.3	3.9	4.6	5.1	5.5	6.2	6.8	7.4	8.2	8.6	9.0
Average diameter of colonies in centimeters . . . . .	0	1.8	3.2	5.6	6.8	7.4	7.6	8.0	7.2	4.6	3.2	2.2

The results show that the pH reaction most favorable for growth was 6.8, which probably is very near the optimum. The organism grew slowly at pH 3.3 and was inhibited at pH 2.5. The maximum reaction for growth was not obtained but it is probably not much higher than pH 10.0, because growth was slow at pH 9.0.

#### Overwintering

Since the fungus does not grow very readily at the lower temperatures, it seems probable that it remains more or less inactive during the winter, depending on moisture and temperature conditions. The fungus overwinters in the form of sclerotia, scattered throughout the soil and in the infected refuse left in cornfields. This also probably aids in dissemination, since the sclerotia may be washed considerable distances across fields. The organism may also live over as dormant mycelium in the infected kernels left in the field. Sclerotia placed in small glass vials and sealed remained viable for 25 days at 0° C., and sclerotia frozen in a cake of ice remained viable for 15 days. Such low temperatures do not occur in Florida in the field, but the above test illustrates the ability of the fungus to tolerate low temperatures and thus remain viable over winter in the field.

#### SUMMARY

A sclerotial dry rot of corn ears in the field is described for the first time. This disease is caused by the fungus *Rhizoctonia zeae*, n. sp. At certain stages it could be easily mistaken for that caused by *Diplodia zeae* or *D. macrospora*. The outstanding symptoms of the disease are discussed.

The fungus is capable of rotting the seedling roots and mesocotyls of corn, but is most important as causing an ear rot. Ears of corn in the early stages of growth proved more susceptible to infection than in the later stages of development.

The morphological characters of the fungus were studied, including the origin and development of the sclerotia.

*Rhizoctonia zeae* grows well and produces numerous sclerotia on standard culture media and on sterilized corn kernels and corn meal.

In pure culture, on potato-dextrose agar, the minimum temperature for mycelial growth for the organism is between 11 and 14° C., the maximum lying between 40 and 42° C. and the optimum near 33° C.

No growth developed on potato dextrose agar at pH 2.5, and the fungus grew best at pH 6.8. The maximum reaction was not determined but it is probably not much higher than pH 10.0.

The fungus hibernates as dormant mycelium and sclerotia in seed and in old plant debris and in the soil.

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# NOTES ON THE CONTROL OF TRANSIT AND STORAGE DECAYS OF TOMATOES BY THE USE OF CHEMICAL WASHES

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## INTRODUCTION

The substantial losses sustained each year by growers and shippers of tomatoes, due to decays developing during transit and storage, have been pointed out by several investigators. In 1918 Shear (7) gave an excellent résumé, with citations to literature, of the losses involved in the fruit and vegetable industry. Rosenbaum (5, 6) and Ramsey and Bailey (2, 3, 4) in subsequent papers have developed and clarified certain phases of the problem as it applies to the tomato industry. Recently, Stevens and Nance (8) have called attention to freight-claim statistics for 1930, which show that "tomatoes stood first in average amount of loss per car lot, with \$40.04 as compared with an average for all commodities of \$11.25."

While stationed in the winter tomato-shipping section of southern Florida, during the winters of 1929 and 1930, the writer conducted a series of experiments to test the efficacy of a number of chemical solutions or treatments, used as washes, in reducing tomato-fruit transit and storage decays. As circumstances have made it impracticable for the writer to continue these studies, the data accumulated during the 2 seasons are here briefly presented.

## PURPOSE OF EXPERIMENTAL WORK

The purpose of these experiments was to determine the comparative effectiveness of a group of chemical washes in controlling transit and storage tomato-fruit rots, as they occurred on large commercial samples of Florida winter tomatoes. In order that these washes might be tested at the same time for control of specific fruit-rot organisms each sample was inoculated with a pure culture of one of three tomato fruit-rot organisms that cause a large proportion of tomato-fruit decays, namely, *Phoma destructiva* Plowr., *Rhizoctonia solani* Kühn or *Phytophthora terrestris* Sherb.

## EXPERIMENTAL METHODS

The procedure followed in all groups of tests was essentially the same. For each group of treatments mature green tomatoes, as received from the field at the packing house, were carefully sorted and all fruits showing breaks in the skins or any indications of infection were discarded. From

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this quantity of carefully selected and cleaned fruits, individual samples of 25 fruits each were counted out for subjection to the various treatments. Each sample was placed in a 4-quart carrier, where it remained during the course of treatment and storage. All except the noninoculated check fruits were then inoculated with a pure culture of either *Phoma*, *Rhizoctonia*, or *Phytophthora*. The fruits were sprayed with an aqueous spore suspension of the *Phoma* culture, while the *Rhizoctonia* or *Phytophthora* cultures were applied by sprinkling the fruits with an aqueous suspension of the shredded fungous mat. In order to allow the inoculum time to adhere to the fruits the inoculated tomatoes were not placed in the washing solutions until after the free water had evaporated from the fruit surfaces. The washes were held in wooden vats and were maintained at temperatures between 115° and 122° F. while in use, as preliminary tests with most of these washes indicated that they were more effective when used hot, and no apparent injury to the fruits occurred at a maximum temperature of 122° F. Fifty fruits (2 4-quart carriers) were submerged in each wash for 3 minutes. Half the fruits of each lot were then stored without rinsing, the other half were rinsed in tap water before they were placed in storage. In each experiment 50 fruits were stored noninoculated, as they were received from the field, and 50 inoculated fruits were stored without any washing treatment as controls. All fruits were held throughout the period of storage on shelves in one storage room.

The storage room was insulated with a 4-inch layer of sawdust over the ceiling, between the sidewalls, and on the floor. An atomized spray of water was maintained in the center of the room and the air was kept stirred by an oscillating fan during the incubation period. No attempt was made to control the temperature of the storage room, but the air-temperature readings of the room were recorded daily for each experiment. The lowest mean temperature at which any wash was tested was 63.9° F. and the highest mean temperature of any experiment was 74.9° F. The temperature fluctuations for all experiments under consideration were, therefore, not excessive, and, since the various treatments were repeated at different times, the mean storage temperatures of the various treatments (Table 1), averaged about 70° F. where 6 or more tests were made.

The relative humidity of the atmosphere of the room was determined in the morning and evening by means of a sling psychrometer. The mean minimum relative humidity for all experiments was 93.3 per cent and the maximum approached the saturation point. The humidity was quite uniform for all experiments and the range closely approximated the daily fluctuations. The humidity of the storage room was maintained at a higher percentage than tomato fruits would normally encounter in transit and storage, in order to favor the development of the various storage decays and to shorten the storage period.

The tomatoes were held in storage from 9 to 17 days, depending on the rapidity with which the decays attacked the fruits, the usual period being 2 weeks. When the storage period was terminated each fruit was examined and the number of fruits having lesions caused by the fungus with which they were inoculated at the beginning of the storage period was recorded separately. The fruits having lesions caused by other organisms were grouped together. Of course, an indeterminate number of lesions in both groups were initiated in the field, but this does not materially affect the validity of the results, since all treatments and controls in any one group of determinations were subjected to the same field conditions.

#### PRESENTATION OF RESULTS AND DISCUSSION

During the 2 seasons this work was in progress, 18 series of tests were conducted in which 8375 tomato fruits were inoculated and treated with 21 different chemical washes. An additional 2200 fruits were used for controls. Table 1 shows the mean percentage of sound fruits obtained in all inoculated rinsed and nonrinsed series at the end of the storage periods by the use of each washing treatment together with the mean percentage of sound fruits found in the corresponding control lots and the mean difference in percentage between them (columns 3, 4 and 5, respectively). The odds of significance shown were computed according to Student's (9, 10) method. In columns 1 and 2 are given the number of tests made with each chemical wash and the mean storage temperature at which all the tests were made.

It will be observed that the odds are significant in every case and are very high for every treatment tested 10 or more times. The average difference in percentage of sound tomatoes over the controls was the highest in those tests where formaldehyde-borax solution was used. An average difference of 59.5 per cent of sound fruit was obtained in the 16 tests. However, the percentage differences of sound fruit obtained with several other washes were nearly as high. The very poisonous mercuric chloride solution was included as a reference point only for obtaining an estimate of the efficacy of the numerous washes of unknown fungicidal and bactericidal value. The average percentage difference of sound fruits obtained with mercuric chloride over the control series was as good as but no better than the best of the nonpoisonous treatments, which indicates that, under these conditions, especially favorable for the development of rot organisms, there is a 45 to 60 per cent decrease in the number of fruits actually infected after treatment with the most effective of the washes used.

The time the fruits are submerged in the wash must of necessity be short for commercial use, since large quantities of fruit must be handled rapidly. Apparently, the infections that have been initiated in the field

TABLE 1.—*Tabulation of the mean percentages of sound tomatoes found at the end of the storage periods of all tests made of each chemical wash as compared with its inoculated controls*

Washing treatments	Number of tests	Mean storage temperature of all tests	Mean percentages of sound fruits at end of storage periods			Odds
			Nontreated inoculated control fruits	Inoculated treated fruits	Difference	
1 to 300 commercial formaldehyde <sup>1</sup> CH <sub>2</sub> O	32	70.4	19.7	73.1	53.4	over 10,000 to 1
1 per cent potash alum K <sub>2</sub> Al <sub>2</sub> (SO <sub>4</sub> ) <sub>4</sub> · 24H <sub>2</sub> O	18	72.1	20.4	70.6	50.2	do
1 per cent sodium alum Na <sub>2</sub> Al <sub>2</sub> (SO <sub>4</sub> ) <sub>4</sub> · 24H <sub>2</sub> O	6	70.0	26.0	74.7	48.7	107 to 1
1 per cent ammon. alum (NH <sub>4</sub> ) <sub>2</sub> Al <sub>2</sub> (SO <sub>4</sub> ) <sub>4</sub> · 24H <sub>2</sub> O	8	67.6	23.6	39.5	15.9	74 to 1
1 per cent borax Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> · 10H <sub>2</sub> O	20	70.4	20.0	55.3	35.3	over 10,000 to 1
1 per cent chloride of lime Ca(ClO) <sub>2</sub>	22	70.9	19.5	62.0	42.5	do
1 per cent laundry bleach NaClO	6	68.8	24.0	72.0	48.0	263 to 1
1 per cent calomel Hg <sub>2</sub> Cl <sub>2</sub>	6	68.8	24.0	71.3	47.3	227 to 1
1 per cent corrosive sublimate HgCl <sub>2</sub> poison	12	71.3	21.7	79.0	57.3	over 10,000 to 1
1 per cent borax and 1 per cent potash alum	16	70.6	21.0	50.0	29.0	do
1 per cent chloride of lime and 1 per cent potash alum	16	70.6	21.0	78.3	57.3	do
1 per cent chloride of lime and 1 per cent borax	16	70.6	21.0	76.3	55.3	do
1 per cent chloride of lime, 1 per cent borax, and 1 per cent potash alum	16	70.6	21.0	74.8	53.8	do
1 per cent potash alum in 1 to 300 commercial formaldehyde	22	70.5	22.4	79.3	56.9	do

TABLE 1—(Continued)

Washing treatments	Number of tests	Mean storage temperature of all tests	Mean percentages of sound fruits at end of storage periods			Odds
			Nontreated inoculated control fruits	Inoculated treated fruits	Difference	
1 per cent ammonium alum in 1 to 300 commercial formaldehyde	4	63.9	31.0	80.0	49.0	31 to 1
1 per cent borax in 1 to 300 commercial formaldehyde	16	70.6	21.0	80.5	59.5	over 10,000 to 1
1 per cent chloride of lime in 1 to 300 commercial formaldehyde	16	70.6	21.0	75.0	54.0	do
1 per cent potash alum and 1 per cent borax in 1 to 300 commercial formaldehyde	10	73.0	16.8	62.4	45.6	do
1 per cent chloride of lime and 1 per cent borax in 1 to 300 commercial formaldehyde	10	73.0	16.8	71.6	54.8	do
1 per cent chloride of lime and 1 per cent potash alum in 1 to 300 commercial formaldehyde	4	74.9	25.0	76.0	51.0	61 to 1
1 per cent chloride of lime, 1 per cent borax and 1 per cent potash alum in 1 to 300 commercial formaldehyde	16	70.6	21.0	79.5	58.5	over 10,000 to 1

<sup>1</sup> One part of 40 per cent commercial formaldehyde to 300 parts tap water by volume. All the other chemicals were used on a weight-volume basis.

before the tomatoes are picked determine to a considerable extent the keeping quality of tomatoes in storage, whether they are surface sterilized or not before the storage period. Wardlaw and McGuire (11), in Trinidad, indicate that the length of time the different lots of tomato fruits remained sound in their storage experiments was influenced greatly by the incidence of infections under field conditions before the fruits were placed in storage. In this connection, the relative amounts of decay produced during these experiments by the 3 fungi, which cause a large proportion of Florida tomato fruit rots, seem worthy of notice. The comparative percentages of decay caused by *Phoma*, *Rhizoctonia*, and *Phytophthora* in the inoculated and noninoculated controls and also in the series of all experiments treated with commercial formaldehyde are listed in table 2.

TABLE 2.—Comparison of the percentages of fruits decayed by three tomato fruit-rot fungi in the inoculated and in the noninoculated controls and in the inoculated series submerged in formaldehyde solution

Fungus	Inoculated controls Average decay	Noninoculated controls Average decay	Inoculated formaldehyde- treated series Average decay
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
<i>Phoma destructiva</i>	39.3	32.0	11.50
<i>Rhizoctonia solani</i>	55.1	1.6	1.25
<i>Phytophthora terrestris</i>	96.0	5.6	1.20

In interpreting these percentages one must keep in mind that they were derived from results obtained with random samples of fruit from 2 crop years and that all 3 fungi were not tested simultaneously but usually at successive intervals of a few days. However, each fungus was tested several times during the 2 seasons. Remembering that each fruit was selected macroscopically as being sound at the beginning of the storage period, note that a relatively high percentage of *Phoma* infections occurred in the noninoculated controls, which indicates a high prevalent incidence of undetectable *Phoma* infections on the fruits as they arrive at the Florida packing houses. On the other hand, the average percentage of infection occurring in the series inoculated with *Phoma* spores at the beginning of the storage period is but little higher than the noninoculated series, which indicates that the source of most of the *Phoma* infections that developed during the storage period must be in the field. These results agree with the negative results obtained by Miss Jamieson (1), when she inoculated tomato fruits having unbroken skins that had not previously been exposed to infection by *Phoma*. It seems evident, however, that during development under

conditions that prevail in Florida tomato fields, *Phoma* becomes sufficiently embedded in the tissues of the stem region of many apparently unblemished tomato fruits to enable it to produce macroscopic lesions as they approach ripeness. It was also significant that a large number of *Phoma* lesions developed despite the best of the washing treatments tested.

Although soil rot, *Rhizoctonia solani*, and buckeye rot, *Phytophthora terrestris*, also known as water spot, were periodically very prevalent in the fields during the 2 seasons that the random samples of tomato fruits were tested, it is apparent that the field-infected fruits were more readily segregated from the noninfected tomatoes, as shown by the low average percentage decay caused by these fungi in the respective noninoculated control series. The most probable reason for this seems to be the rapidity with which lesions of these fungi develop after infection takes place as contrasted with the apparently imperceptible development of *Phoma* until the tomato fruit approaches the ripening stage.

A comparison of the rinsed and nonrinsed series of all washes in all experiments for the 2 years indicates that rinsing the fruits in tap water immediately after they were removed from the washes slightly decreased the average percentage of sound fruits found at the end of the storage period. The difference obtained was, however, insignificant for all washes except those containing formaldehyde in combination with other chemicals, where a small significant difference was obtained in favor of the nonrinsed series (Table 3).

TABLE 3.—Comparison of the percentages of sound tomato fruits found at end of storage periods for all the rinsed and the nonrinsed series of grouped washes

Groups of washes	Nonrinsed series, sound fruits	Rinsed series, sound fruits	Difference, sound fruits	P.E.M.
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
Washes containing formaldehyde in combination with other chemicals . . . . .	79.03	69.65	9.38	± 1.40
Washes containing formaldehyde only . . . . .	77.85	74.46	3.69	± 1.26
Washes containing no formaldehyde . . . . .	67.43	63.62	3.81	± 3.54

Notwithstanding the small consistent differences in favor of the non-rinsed series of all 3 groups of washes it seems inadvisable to recommend leaving the washes on the fruit surfaces unless the advantage is more conclusively shown by further investigation. Although there was no apparent injury to the tomatoes due to any of the washing treatments as used in

the tests recorded in table 1, and they ripened normally with the controls, the nonrinsed fruits would have to have the residues left by the chemical washes removed before they could be marketed.

From time to time interested persons have expressed the opinion that treating tomato fruits with ethylene gas improved their keeping qualities. In order to test this opinion, groups of tomatoes inoculated with *Phoma*, *Rhizoctonia*, and *Phytophthora* and one noninoculated group were kept for 3 successive days in a room where a cubic foot of ethylene gas per 1000 cubic feet of air was liberated each day. Nongassed inoculated and noninoculated groups of tomatoes were kept in an adjoining room for 3 days. All gassed and nongassed groups were then transferred to the storage room. The percentages of decay occurring at the end of 2 weeks in each group of 50 fruits each are recorded in table 4.

TABLE 4.—*Effect of exposure to ethylene gas for 3 days on the development of tomato fruit decays*

Infecting fungus	Decay in groups not treated with ethylene gas	Decay in groups treated with ethylene gas
	<i>Per cent</i>	<i>Per cent</i>
<i>Phoma</i>	20	25
<i>Rhizoctonia</i>	78	80
<i>Phytophthora</i>	100	100
Noninoculated	42	36

It is apparent that the ethylene treatment had no significant effect on the development of tomato fruit rots in any of these groups.

#### SUMMARY

The relative effectiveness of a group of chemical washes in inhibiting tomato fruit rots was tested on commercial samples of "green-wrap" Florida winter tomatoes in storage. Submerging inoculated tomato fruits for 3 minutes in hot washes before placing in storage reduced the number of decayed fruits found at the end of the storage periods an average of 29 per cent for the least effective wash, and an average of 59.5 per cent for the most effective wash used. Rinsing of the fruit immediately after treatment did not significantly increase the number of fruits developing rots in storage, except in the groups of fruit washed in formaldehyde solutions containing other chemicals, where there was a small significant increase in percentage of decayed tomatoes. Most of the *Phoma* infections that developed on the tomato fruits while in storage were natural infec-

tions that could not be detected when the tomatoes were stored, and part of them could not be inhibited by the most effective washes tested. Ethylene gas as used for accelerating the coloring of tomato fruits had no effect on the development of tomato fruit rots.

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## PHYTOPATHOLOGICAL NOTES

*The Antiquity of Puccinia Graminis and Ustilago Tritici in California.*—In 1880 Harkness and Moore<sup>1</sup> published a list of some 860 species of fungi that they had collected mainly in California in 1876, 1877, 1878, and 1879, and their inclusion of *Puccinia graminis* Pers., in this list, recorded as occurring “On wheat and sugarcane.<sup>2</sup> San. Fran. and S. Is.<sup>3</sup>”, is thought to constitute the earliest definite record of that organism in the Pacific Coast region. *Ustilago tritici* (Pers.) Rostr. does not appear in this list and is thought to have been first collected in the Pacific Coast region at a still later date. It is our purpose in publishing this note to call attention to new evidence regarding the antiquity of these 2 organisms in California and to suggest the wider applicability of the method we have employed to the study of the history of parasitic fungi in general in the region under consideration.

Since 1925, Hendry,<sup>4, 5</sup> has given attention to the fragments of higher plants that are a common constituent of sun-dried earthen bricks composing the walls of historic adobe buildings, and by this means has established the identity of numerous wild and cultivated plants, in the localities studied, that were present concurrently with the erection of such buildings. Recently, in the pursuit of these studies, it has become apparent that such higher plants have also occasionally served as hosts for fungi, and that occasionally such organisms may be specifically identified. In the examination of a collection of such plant remains, consisting principally of wheat straw taken from the interior of sun-dried earthen bricks collected from the ruins of the church building of the Mission Nuestra Senora De La Soledad, erected in the central Salinas Valley of California during the years 1793 and 1794, but rebuilt in 1832, following destruction by flood, one of us (Hansen) has identified both *Puccinia graminis* Pers. and *Ustilago tritici* (Pers.) Rostr. Teliospores and urediospores of the former, on both the culm and rachis of Little Club wheat, *Triticum compactum humboldtii*, Kecke., and spores of the latter lodged in the tufts of rachis hairs of the above named wheat.

<sup>1</sup> Harkness, H. W. and J. P. Moore, “Catalogue of the Pacific Coast Fungi.” California Academy of Sciences, unnumbered pamphlet, Feb. 2, 1880.

<sup>2</sup> Sorgo, *Andropogon Sorghum* var. *saccharatus*, Alef., was under trial in California in the late 70's and early 80's as a possible source of sugar and was then commonly referred to as “Chinese Sugar Cane” or as “Sugar Cane.”

<sup>3</sup> May refer to Sandwich (Hawaiian) Islands, in which case the host was probably true sugar-cane, *Saccharum officinarum*, L.

<sup>4</sup> Hendry, G. W. and M. P. Kelly. The plant content of adobe bricks, Quart. Calif. Hist. Soc. 4: 361-373. 1925.

<sup>5</sup> Hendry, G. W. The adobe brick as a historical source. Reporting further studies in adobe brick analysis. Agr. Hist. 5: 110-127. 1931.

These finds prove the existence of these organisms on the host mentioned in the vicinity of Soledad in the year 1832, antedating by more than 40 years all records known to us for the occurrence of these organisms in California.

We are now studying older material from this and other historic edifices that promises to yield a further extension of our knowledge of the history of pathogenic fungi and their hosts in this region.—G. W. HENDRY and H. N. HANSEN, University of California, Berkeley, California.

*Some Pathological Observations on Sugar Cane × Sorghum Hybrids in Florida.*—The first cane × sorghum hybrids produced in Florida were in 1931–1932,<sup>1</sup> when the sugar cane variety POJ.2725 was crossed successfully with Texas Seeded Ribbon and Early Orange, varieties of *Holcus sorghum* var. *saccharatus* Bailey. POJ.2725 was first crossed successfully with *Sorghum durra* Stapf by Thomas and Venkatraman<sup>2</sup> in India. While mention has been made of various morphological and structural characters of leaves, stems, and flowers of the resulting F<sub>1</sub> progeny of this cross; there is no published record of the occurrence of diseases among the hybrids.

During a detailed study of the inheritance of certain leaf, stem, and floral characters among 58 hybrids between POJ.2725 and Texas Seeded Ribbon sorghum, notes also were made of the occurrence on them of mosaic and certain common leaf and sheath diseases frequently attacking sugar cane and sorghum.

Although POJ.2725 has been reported to show occasional infection with mosaic in certain regions of Cuba,<sup>3</sup> it has never been known to become infected with this disease in Florida, even with thousands of acres under commercial culture and exposed to infection. POJ.2725 is nearly immune from red rot of the sheath (*Colletotrichum falcatum* Went.) but, depending on soil and location, it is, however, more or less susceptible to eye spot (*Helminthosporium ocellum* Faris), brown stripe (*Helminthosporium stenospilum* Drechsler) and brown spot (*Cercospora longipes* Butler).

While the sorghum variety, Texas Seeded Ribbon, has frequently been found infected with mosaic when growing very close to the hybrid plots, it never has been found infected with any of the other diseases occurring on sugar-cane leaves or sheaths. One of the commonest diseases present on the leaves of this sorghum host was the leaf rust (*Puccinia purpurea* Cooke).

<sup>1</sup> Bourne, B. A. Cane breeding experiments. Florida Agr. Exp. Sta. Ann. Rpt. 1932: 205–207. 1932.

<sup>2</sup> Thomas, R. and T. S. Venkatraman. Sugarcane-sorghum hybrids. Agr. Jour. Ind. 25: 164. 1930.

<sup>3</sup> Stahl, C. F. and James A. Faris. The behavior of the new POJ canes in relation to sugar cane mosaic in Cuba. Trop. Plant Res. Found. Bul. 9: 1929.

A summary of observations to date on the susceptibility of the hybrid progeny to 4 diseases shows that approximately two-thirds of the hybrids are susceptible to the 2 *Helminthosporium* diseases of sugar cane, one-third to brown spot (*Cercospora longipes*) and one-fourth to the red rot of the sheath (*Colletotrichum falcatum*).

So far, none of the progeny of the above cross or any other hybrids between POJ.2725 and varieties of *Holcus sorghum* var. *saccharatus* have been found infected with mosaic. However, the results of 2 years' observations should not be considered final, as certain varieties of canes that are normally immune from mosaic on Okeechobee muck lands develop the disease when grown on relatively raw peat soils (willow and elder type) high in available nitrogen and low in total mineral content, especially silica.

The absence of leaf rust (*Puccinia purpurea*) among the hybrid progeny is also of special interest, since all of the locally grown varieties of *Holcus sorghum* var. *saccharatus* readily become infected with this fungus.—B. A. BOURNE, Florida Agricultural Experiment Station, Gainesville, Florida.

*Felicia amelloides* Voss, an Unreported Host for *Erysiphe cichoracearum* DC.—The blue Agathea daisy, popular both as an outdoor garden flower and for potting or winter cutting in greenhouses, has been found attacked rather severely by *Erysiphe cichoracearum* DC. in Rhode Island. No report of a powdery mildew on this host, *Felicia* (*Agathea*) *amelloides* Voss, could be found, although the fungus has been reported on various other members of the Compositae.

A group of diseased plants growing in a bench in the college greenhouses was called to the writer's attention in the past winter. The lower leaves, especially, were covered with the white mycelium and conidiophores of the mildew. Also many scattered patches of dark reddish brown perithecia were present. Infected leaves became dry, died prematurely, and dropped off. The perithecia are typical of *Erysiphe cichoracearum*; each contains 8 or more asci and each more or less stalked ascus usually contains 2 ascospores. The haustoria are simple. A limited amount of material is available for distribution to herbaria.—FRANK L. HOWARD, Rhode Island State College, Kingston, Rhode Island.

## BOOK REVIEW

Arthur, Joseph Charles. *Manual of the Rusts in United States and Canada*. ii-xv + 438 pp. 487 figs. Illustrations by George B. Cummins. Purdue Research Foundation, Lafayette, Indiana. 1934.

Unfortunately, the general mycologist and plant pathologist has found it difficult to utilize the monograph of the rusts of North America published in the North American Flora (N. Am. Flora 7: 85-969). This is partly due to use of life cycles in the separation of genera, with the resulting employment of unfamiliar names for the most common plant pathogens, names that have found little or no use in the literature of plant pathology and mycology. It is partly due to the difficulty of using a monograph published in parts over a period of 20 years with the resulting changes in concepts, classification, and terminology that naturally occur with accumulating knowledge. It therefore is fortunate that Dr. Arthur has incorporated the results of his years of intensive study of the rusts in a compact manual in the preparation of which one of the main objects has been "to produce a work to be serviceable in the determination of collections of rusts by the general botanist."

In the *Manual of the Rusts in United States and Canada*, life cycles have not been employed in the separation of genera. As a result, the classification is much simpler and easier to use and, what is probably of more importance to the general pathologist, the names employed are for the most part those in general usage. Also, in so doing, another object of the manual, the presentation of a classification showing relationship of species and genera, has been furthered.

The preface deserves careful study, since in it several innovations are discussed that should be clearly understood if the manual is to be utilized without confusion. Considerable emphasis is placed on the relationship of the various spore forms to the gametophytic (haploid) and sporophytic (diploid) states. Pycnia and aecia are considered the products of the gametophyte and uredia and telia of the sporophyte. As the term is employed in the manual, an aecium is the diploid spore form produced by the gametophytic mycelium of a macrocyclic rust. On this basis the sori containing pedicellate spores accompanying the pycnia of a brachypuccinia (primary uredinia of the N. Am. Flora) are uredinoid aecia, while the similar sori resulting from them (secondary uredinia of the N. Am. Flora) are uredia. Likewise, in some opsis rusts, infection from aeciospores may produce aecidia which in the terms of the manual would be aecidioid uredia (secondary aecia of the N. Am. Flora). Since the terms aecium, etc., are not based upon morphology, which is indicated by the modifying adjectives, it is unfortunate that such adjectives are used in only a few species. Apparently, in all cases, uredinoid aecia are so designated. However, in most of the species only the unmodified term aecium is employed. It is left for one to assume that these have catenulate aeciospores, produced in

aecidioid aecia. The terms uredia and urediospores are used to replace uredinia and urediniospores (Ber. Deutsch. Bot. Gesellsch. 50a: 25. 1932). It is somewhat questionable whether any important advantage is derived.

On the basis of life cycle the rusts are divided into macrocyclic, having all spore forms or with uredia lacking (demicyclic) and microcyclic, having telia with or without pycnia. The latter are considered reduced forms.

In general, the International Code of Nomenclature has been followed with the exception that "the date for beginning of nomenclatural priority is taken to be 1753, not 1801." It is stated that this change affects only 5 specific names, resulting in the retention of names in common usage. A discussion of the phase "perfect state" of the International Code is given, in which it is maintained that this should be interpreted to apply to the sporophytic state and that, therefore, *Uredo* names are permissible. Such an interpretation, of course, would exclude *Uredo* names applied to uredinoid aecia.

Two families are recognized, the Melampsoraceae, which is divided into 4 tribes—Pucciniastreae, Cronartieae, Melampsoreae and Phakopsoreae—and the Pucciniaceae with 3 tribes—Raveneliae, Phragmidiae, and Aecidiiae. The Melampsoraceae are characterized by sessile teliospores, the Pucciniaceae by pedicellate teliospores.

The genera *Uredinopsis*, *Milesia*, *Hyalospora*, *Pucciniastrum*, *Melampsorella*, *Melampsoridium*, *Cronartium*, *Chrysomyxa*, *Coleosporium*, *Melampsora*, *Aplopsora*, *Bubakia*, *Physopella*, *Cerotelium*, and *Baeodromus* are treated in the Melampsoraceae. For the genus *Milesia*, Faull's recent monograph is followed. *Thecopsora* and *Calyptospora* are treated as sections of the genus *Pucciniastrum*.

The genera *Ravenelia*, *Pileolaria*, *Tranzschelia*, *Cumminsiella*, *Uropyxis*, *Phragmopyxis*, *Phragmidium*, *Xenodochus*, *Frommea*, *Kuehneola*, *Mainsia*, *Gymnoconia*, *Trachyspora*, *Triphragmidium*, *Nyssopsora*, *Puccinia*, *Uromyces*, and *Gymnosporangium* are discussed in the Pucciniaceae. Species of *Uromyces* are not treated as a separate group, but are distributed with species of *Puccinia* according to their relationship. The genus *Puccinia* is divided into 2 sections, *Eupuccinia* and *Bullaria*. The statement concerning the separation of the 2 sections is somewhat misleading. It is stated (p. 111) that the aecia of the species belonging to the section *Bullaria* are uredinoid with echinulate aeciospores, less often aecidioid with verrucose aeciospores. This statement, which would hold for the genus *Bullaria* as used in the North American Flora, does not agree with the data given for the species placed in the section *Bullaria*, since most of these have aecidioid aecia and verrucose aeciospores. The separation, which seems to be a logical one, is determined principally by the shape and character of the wall of the teliospores. Under the heading of Form-genera, unconnected stages are placed in *Aecidium*, *Peridermium*, *Caeoma*, *Uraecium* (uredinoid aecia), and *Uredo*.

Keys to the genera are given under each family and to the species under each genus. In the keys to species, correlated species, although retaining their specific identity, are listed, following the most fully developed species. Only the latter is given a number. Demicyclic and microcyclic species are indicated by the designations (d) and (m), respectively. Species of *Uromyces* and *Endophyllum* are similarly placed. By this means, closely related species are grouped together. Each numbered group of species "may in a way be considered to represent an ancestral species broken into more or less distinct forms, now called correlated species, during evolutionary development." This interesting innovation has the advantage of emphasizing the close relationship of groups of species that, in most treatments of the rusts, are widely separated when arranged on the basis of hosts or are placed in separate genera because of differences in life cycle or morphology.

The specific descriptions are concise, but include important diagnostic characters. Only the most important synonyms are listed. References, however, are given to the North American Flora where more detailed information is available. The descriptions are accompanied by 487 excellent text figures by George B. Cummins. These greatly aid in visualizing the species and will be an important help in identification. A very valuable addition to the information concerning the species is a statement giving the results of cultures. This enables one to evaluate the assignment of hosts for the various stages and probably will stimulate studies in this field.

As stated in the preface, principal emphasis is placed upon morphologic differences in the separation of species. This has resulted in placing in synonymy a number of species that have been separated largely on the basis of host specialization. Most important in connection with the literature of plant pathology is the inclusion of *Puccinia triticea* and *Puccinia dispersa* as varieties in the species *Puccinia rubigo-vera*.

In a number of species, varieties are distinguished, part on a morphologic basis, part by host specialization, and part by geographic distribution. In a few cases it is somewhat questionable whether the distinctions made are really significant. Thus, *Puccinia rubigo-vera agropyri* and *P. rubigo-vera agropyrina* are apparently separated principally on the basis of range. They include groups, that can be distinguished much better by morphology and host specialization. Groups thus separated would conform more closely to the other varieties that are recognized in the species. No attempt is made to include the results of the extensive studies of physiologic specialization in the cereal rusts.

The Manual will unquestionably be very useful, not only to the general botanist but to the beginning student and the specialist, as well. It not only will serve as a very convenient and useful reference but also will clarify the taxonomic relationships of North American species.—E. B. MAINS, University of Michigan, Ann Arbor, Michigan.

# PHYTOPATHOLOGY

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## MICROORGANISMS SURVIVING THE STORAGE PERIOD OF FROZEN-PACK FRUITS AND VEGETABLES

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### INTRODUCTION

The preservation of foods by freezing is a practice of long standing, but the practical application of freezing preservation to fresh fruits and vegetables is of rather recent development. It is believed that the earliest report in the scientific literature of practical experiments on the freezing of such products is contained in Bulletin No. 108 of the Bureau of Plant Industry, published in 1907 by S. H. Fulton (6), which describes experiments on freezing of strawberries and other small fruits. Shortly thereafter the preservation of berries in the Pacific Northwest was undertaken, and by 1928 the industry had developed from a few hundred barrels in the early years to about 100,000 barrels of 50-gallon capacity (4).

About 5 years ago small consumer packages of frozen fruit and vegetables were put on the market, and today a considerable variety of these products is available in this form. Their introduction raised the question of their healthfulness and made desirable the investigation of the microbiology of frozen-pack foods. This was undertaken by the Bureau of Plant Industry of the United States Department of Agriculture in 1929, and the present report deals with some of the findings from this work.<sup>1</sup>

Some 10,000 containers of frozen fruit and vegetables, (including experimental and commercial packs,) have been examined superficially for appearance, texture, taste, etc., while careful microbiological analysis has been made on not less than 3,000 samples of the available material. The data presented have been collected from frozen products packed in 50-gal. barrels, tin cans holding 5 or 10 gallons, No. 2 sealed tin cans and various kinds of glass jars and paper containers. Some of the tin cans and glass containers were sealed under vacuum.

One part of the present work has been to determine through repeated examinations of material the numbers and types of bacteria, yeasts and fungi commonly found in fruits and vegetables frozen in different ways.

<sup>1</sup> The writer wishes to express appreciation to Dr. C. A. Magoon, Senior Bacteriologist, Bureau of Plant Industry, under whose direction this work was done.

The subject has been treated from the viewpoint that an environment long recognized as interesting, because of a change in commercial practises, has assumed a new importance calling for exacting study. It was thought necessary also to determine the microbial flora of the fresh fruits and vegetables going into frozen-pack products, and some additions to the knowledge of ecological bacteriology have thus been made. Microorganisms growing under aerobic conditions are the only types considered in this report.

Frozen-pack material has been studied from three of the important packing centers in the country, namely, the Pacific Northwest; the eastern district centering around Norfolk, Virginia; and the southern or Louisiana district. Frozen-pack strawberries are the most widely used of any of the frozen fruits and a large part of the experimental data presented here has to do with this product. The data on strawberries will be followed by reports on other small fruits and, finally, a record of the work done on frozen pack vegetables will be given.

It has not been found feasible in all cases to determine the identity of some of the fungi and yeasts. Generic names are, at present, all that can be given in most cases. The bacteria have been identified as belonging to well-known groups but in many instances the purified cultures, when run through the prescribed tests, failed to conform in all respects to the standard reactions of species to which they obviously belonged. This failure in all probability is to be explained on the ground of disturbed metabolism resulting from the unfavorable conditions that prolonged low temperature storage of fruits and vegetables imposed or to normal variation in biochemical reactions within a bacterial species.

#### METHODS

One hundred grams of the material to be analyzed was measured into a sterile jar and mashed thoroughly. Sterile water to the amount of one hundred cubic centimeters was added and the mixture thoroughly agitated to bring about an equal distribution of the microorganisms. Proper dilutions were made and plates poured, using both standard beef agar at pH 7.0 and wort agar,<sup>2</sup> which, having a distinctly acid reaction and a higher sugar content, favors a better growth of fungi and yeasts.

The colonies of organisms on the beef-agar plates were counted at the end of 48 hours, while the wort-agar plates were incubated 4 or 5 days to allow the fungi to sporulate. The predominating types of microorganisms in all samples were noted and cultures from them made for study and identification.

<sup>2</sup> Wort agar formula—Hop-flavored malt syrup ... 100 g.  
Distilled water ... 1,000 “  
Agar ... 15 “ } reaction about 4.6 pH



Every bacterial form so selected was subjected to all the standard morphological, cultural, and physiological tests prescribed by the Society of American Bacteriologists in their Manual of Methods (9). With these tests completed and using Bergey's Manual of Determinative Bacteriology (1) and Ford's Textbook of Bacteriology (5), the cultures were identified as closely as possible.

#### EXPERIMENTAL DATA ON STRAWBERRIES

A wide variation in the numbers of microorganisms was found on fresh strawberries depending on their condition when samples were taken. Some of the berries going into frozen packs were fresh, firm, and clean; others were over-ripe, spotted with decay, and had stood several hours before packing. Samples of the same lots of fruit after a year's storage at 15° F. showed a remarkable reduction in the number of viable organisms, amounting in some cases to practical sterilization. The fact that defrosted berries make an ideal culture medium for the growth of many microorganisms and that some undesirable and even dangerous forms may survive the freezing period make it necessary to find out exactly how many and what kinds of microorganisms are to be found in frozen packs and how they affect the quality and healthfulness of the defrosted product.

Table 1 shows the numbers of microorganisms per gram of representative samples of fresh-washed strawberries from the 3 packing centers. This table also gives the numbers of microorganisms surviving a year's storage

TABLE 1.—*Microbial content of fresh washed strawberries and of the same lots of berries after 1 and 3 years' storage in sealed tin cans and other containers at 15° F.*

Locality	Microorganisms per gram of fresh strawberries	Count per gram same lot of strawberries after 1 yr. at 15° F.	Percentage reduction in viable organisms	Count per gram same lot of strawberries after 3 yrs. at 15° F.
Northwest	14,500	525	96.3	245
"	30,500	90	99.7	32
"	1,030,000	290	99.9	50
Eastern	320,000	170	99.9	..
"	495,000	270	99.9	..
"	615,000	130	99.9	..
Southern	25,500	330	98.7	..
"	31,500	190	99.3	..
"	67,500	170	99.7	..
"	230,000	100	99.9	..
Average	.....	.....	99.3	..

of the same lots of fruit in sealed tin cans and other containers and in the case of some samples of fruit from the Pacific Northwest, 3 years' storage at freezing temperatures. The decrease in numbers of viable microorganisms is seen to be very great. There is reason to believe that rapid freezing at very low temperatures does not have the same sterilizing effect. [See Berry (3).]

The reduction in microbial content of strawberries held in sealed tin cans and other containers for a year at 15° F., as shown in this table, amounts to an average of 99.3 per cent, but the few surviving microorganisms may increase rapidly as soon as the product is defrosted, causing it to mold or ferment, depending on the type of organism gaining the ascendancy in the berries. It should be pointed out that low microbial content does not necessarily mean a high grade frozen product; other factors, such as variety of berry, method of packing, freshness of the fruit when packed, type of container, and proper control of temperature are all highly important in determining its quality.

Since the final spoilage of the frozen product is brought about by the organisms surviving the storage treatment, it was thought desirable to know what types of viable organisms remained in the frozen berries.

Table 2 gives an alphabetically arranged list of the microorganisms that were repeatedly isolated from fresh and frozen strawberries. Those occurring on the fresh fruit are shown in the first column and those surviving the freezing treatment and storage, in the second column.

This list of microorganisms isolated from fresh strawberries shows a wide range of genera, which includes, in the case of the bacteria, some that are undoubtedly of animal or human origin, such as *Aerobacter aerogenes*, *Staphylococcus albus*, *Staph. aureus*, etc. Although none of the organisms listed as isolated from the frozen fruit are known to be pathogenic, the presence of such organisms in the fresh material nevertheless suggests the need for especial care in the sanitary handling of raw material going into frozen storage.

The list of microorganisms found on fresh strawberries would undoubtedly be somewhat longer if more samples had been taken. It will be noted that some species of bacteria are reported from the frozen berries that do not appear in the list of species found on the fresh fruit. This is due in part to the fact that samples of fresh berries were taken only at intervals during the packing, while the entire pack of the frozen fruit was analyzed; and further to the fact that some types of bacteria, yeasts, and molds probably were introduced into the frozen strawberries with the sugar or syrup. Owen (7) reported the occurrence of several species of bacteria in sugar, namely *Bacillus cohaerens*, *B. megatherium*, *B. mesentericus fuscus granulatus*, *B. prausnitzii*, *B. vulgatus*, etc. It will be noted that some of these

TABLE 2.—*Microorganisms commonly found on fresh and frozen strawberries from western, eastern, and southern United States*

Fresh fruit	Frozen fruit
<b>Bacteria</b>	
<i>Achromobacter acidum</i> (Conn) Bergey <i>et al.</i>	<i>Achromobacter acidum</i> (Conn) Bergey <i>et al.</i>
“ <i>album</i> (Copeland) “ “ “	“ <i>butyri</i> (Grimm) “ “ “
“ <i>butyri</i> (Grimm) “ “ “	“ <i>delmarvae</i> (Smart)
“ <i>delmarvae</i> Smart	“ <i>inunctum</i> (Pohl) “ “ “
<i>Acrobacter aerogenes</i> group	“ <i>healii</i> (Buchanan & Hammer) Bergey <i>et al.</i>
<i>Bacillus albolactis</i> (Loëffler) Migula	“ <i>hyalinum</i> (Jordan) Bergey <i>et al.</i>
“ <i>centrosporus</i> Ford	“ <i>venosum</i> (Vaughan) Bergey <i>et al.</i>
“ <i>cereus</i> Frankland	“ <i>viscosum</i> (Adametz) Bergey <i>et al.</i>
“ <i>cohaerens</i> Gottheil	<i>Bacillus albolactis</i> (Loëffler) Migula
“ <i>ellenbachensis</i> Stutzer	“ <i>aterrimus</i> Lehmann and Neumann
“ <i>fluorescens</i> Ford	“ <i>cercus</i> Frankland
“ <i>fusiformis</i> Gottheil	“ <i>coagulans</i> Hammer
“ <i>megatherium</i> de Bary	“ <i>ellenbachensis</i> Stutzer
“ <i>mycoides</i> Flügge	“ <i>fluorescens</i> Ford
“ <i>niger</i> Migula	“ <i>fusus</i> Batchelor
“ <i>parvus</i> Neide	“ <i>graveolens</i> Gottheil
“ <i>prausnitzii</i> Trevisan	“ <i>lactis</i> Flügge
“ <i>silvaticus</i> Neide	“ <i>lobatus</i> Bergey
“ <i>simplex</i> Gottheil	“ <i>mesentericus</i> Trevisan
“ <i>terminalis</i> Migula	“ <i>mycoides</i> Flügge
“ <i>tumescens</i> Zopf	“ <i>platychoma</i> Gray and Thornton
<i>Flavobacterium annulatum</i> (Wright) Bergey <i>et al.</i>	“ <i>polymyxa</i> (Prazmowski) Gruber
“ <i>aquatilis</i> (Frankland) Bergey <i>et al.</i>	“ <i>prausnitzii</i> Trevisan
“ <i>butyri</i> Bergey <i>et al.</i>	“ <i>ruminatus</i> Gottheil
“ <i>caudatum</i> (Wright) Bergey <i>et al.</i>	“ <i>silvaticus</i> Neide
“ <i>flavescens</i> (Pohl) Bergey <i>et al.</i>	“ <i>subtilis</i> (Ehrenberg) Cohn
“ <i>flavum</i> (Fuhrmann) “ “ “	“ <i>tumescens</i> Zopf
“ <i>lutescens</i> (Lustig) “ “ “	“ <i>vulgatus</i> Trevisan
<i>Micrococcus ochraceus</i> Rosenthal	<i>Micrococcus pikowskyi</i> Bergey <i>et al.</i>
<i>Sarcina citrea</i> (Migula) Bergey <i>et al.</i>	<i>Spirillum amyliiferum</i> Van Tieghem
“ <i>flava</i> de Bary	“ <i>virginianum</i> Dimitroff
“ <i>hamaguchiae</i> Saito	<i>Vibrio</i> sp.
“ <i>lutea</i> Schröter	
<i>Spirillum volutans</i> Ehrenberg	
<i>Staphylococcus albus</i> Rosenbach	
“ <i>aureus</i> Rosenbach	
<i>Sphaerotilis</i> sp.	
<i>Proteus</i> like <i>vulgaris</i> Hauser	
<b>Yeasts</b>	
<i>Saccharomyces</i> sp.	<b>Yeasts</b>
<i>Torula colliculosa</i>	<i>Saccharomyces unisporus</i>
A black yeast	
<b>Molds</b>	
<i>Alternaria</i> sp.	<b>Molds</b>
<i>Aspergillus</i> sp.	<i>Aspergillus</i> sp.
<i>Botrytis</i> sp.	<i>Cladosporium</i> sp.
<i>Cladosporium</i> sp.	<i>Dematiu</i> sp.
<i>Fusarium</i> sp.	<i>Oidium</i> sp.
<i>Monilia</i> sp.	<i>Monilia</i> sp.
<i>Mucor</i> sp.	<i>Penicillium</i> sp.
<i>Oidium</i> sp.	<i>Rhizopus</i> sp.
<i>Penicillium</i> sp.	
<i>Rhizopus</i> sp.	
<i>Stemphylium</i> sp.	

species were isolated from fresh strawberries in this experiment while two of them, *B. mesentericus* and *B. vulgatus*, appear only in the frozen berries and may have been introduced with sugar or syrup.

The microorganisms listed above are the types that were repeatedly found in fresh or frozen strawberries in numbers sufficient to warrant their study. The predominant types of bacteria on both the fresh and frozen berries showed some variation in distribution, according to the locality in which the berries were grown. For instance, *Bacillus mycoides* was one of the outstanding species from all localities and appeared in great numbers on fresh strawberries and in lesser numbers in the frozen berries. *B. mycoides*, *B. fluorescens*, nearly all the commoner molds, and many varieties of yeasts were the predominating microorganisms on fresh strawberries in the west and south. The predominating type of bacteria found in all samples of fresh strawberries studied in Maryland, Delaware, and Virginia, in 1929 and 1930, was found to be a new species and was described as such at the annual meeting of American Bacteriologists at Baltimore in 1931. The name *Achromobacter delmarvae* was suggested. A condensed description of this species will be found in the Journal of Bacteriology (8). Very few rods of this species survived one year's storage in strawberries at 15° F.

The types of bacteria found in greatest numbers on frozen strawberries from a given locality remained rather constant. *Bacillus mycoides* was nearly always present, as was *B. silvaticus*, *B. cereus*, *B. mesentericus*, *B. subtilis*, and *B. platychoma*. Among the molds, *Penicillium* probably was the commonest type present in frozen strawberries. Several types of yeasts were found on all the plates.

A surprising number of bacterial species survived 3 years' storage in strawberries in sealed tin cans at 15° F., *Bacillus atterimus*, *B. mycoides*, *B. lobatus*, *B. cereus*, *B. fluorescens*, and *B. subtilis* being the predominating types on most of the plates. *Penicillium*, both an apple-green and a blue-green species, many other molds, and many yeasts were isolated frequently from western strawberries held in sealed tin cans at 15° F. for 3 years.

Molds and yeasts were responsible for spoilage in defrosted frozen-pack strawberries. Generally a slight growth of surface mold could be seen on the strawberries in 2 or 3 days at room temperature. Following this, in most packs, there was an active fermentation caused by yeast. In many samples fermentation started without a preliminary growth of mold, and some packs showing a rather heavy surface growth of mold were not followed by gaseous fermentation.

Most other frozen small fruits showed a wide variety of bacterial species

immediately on defrosting, these being followed by the molds and yeasts that rapidly developed, and thereby destroyed the quality of the product.

#### EXPERIMENTAL DATA ON OTHER FROZEN PACK SMALL FRUITS

In addition to strawberries, which made up the bulk of the material studied, analyses were made of many samples of 6 kinds of other frozen-pack small fruits. Samples of the original material going into these packs were not available for study, consequently the data are only on the frozen products after storage in sealed tin cans and other containers for 1 year at 15° F. Those microorganisms appearing repeatedly on the plates in fairly large numbers were the only ones studied, which makes these lists much shorter than those of species appearing on strawberries.

Frozen blackberries, red currants, and figs had remarkably few viable microorganisms, the counts averaging about 50 per gram. Loganberries had an average count of 200 per gram, raspberries 700, and cherries 2,250 per gram when they were defrosted.

Table 3 shows a list of the bacteria, yeasts, and molds repeatedly found in the above-mentioned frozen products. It will be noted from this list that a great many nonspore-forming types of bacteria are represented as surviving the storage period at freezing temperatures, types generally thought of as being rather sensitive to extremes of temperature or other unfavorable conditions. It is true that some of the vegetative forms of bacteria have unusual powers of resistance, and it is known that very low temperatures fail to kill all microorganisms in heavily inoculated materials of various kinds. However, the presence of *Aerobacter aerogenes* and *A. cloacae* in the frozen figs emphasizes again the necessity for extra care in the preparation of these frozen foods.

Molds and yeasts were the important factors in bringing about spoilage in these fruits, as well as in strawberries. In the case of frozen-pack loganberries, the plates poured from samples immediately after defrosting showed a large number of colonies of *Bacillus mycoides* with a few colonies of *Penicillium* sp. and a smaller number of colonies of yeast. Plates poured from samples taken three days later showed *Penicillium* sp. in greatest numbers, yeasts second in importance, and a very few colonies of *B. mycoides*. Plates poured a day or two later showed almost nothing but yeasts. This sequence of growth was very striking and clear-cut in the case of loganberries. It was less so in some of the other small fruits; for instance, in cherries where a great many yeasts appeared on the plates poured from the first samples taken. It was noticed that frozen blackberries had a rather low microbial content and that it took many days for fermentation to become noticeable.

TABLE 3.—Microorganisms commonly found in six kinds of frozen pack small fruits after storage in sealed tin cans and other containers for 1 year at 15° F.

Frozen product	Average number microorganisms per gram	Bacteria	Yeasts	Molds
Blackberries	40	<i>Bacillus atterrimus</i> Lehmann & Neumann " <i>platychoma</i> Gray and Thornton " <i>mycoides</i> Flügge " <i>sitaticus</i> Neide " <i>subtilis</i> (Ehrenberg) Cohn <i>Spirillum volutans</i> Ehrenberg	<i>Saccharomyces</i> sp.	<i>Aspergillus</i> sp. <i>Penicillium</i> sp.
Cherries ....	2,250	<i>Aerobacter a-rogenes</i> (Kruse) Beijerinck " <i>cloacae</i> (Jordan) Bergey et al. <i>Bacillus mycoides</i> Flügge <i>Flavobacterium rignensis</i> Bergey et al. " <i>tremelloides</i> (Schottelius) Bergey et al. <i>Serratia marcescens</i> Bizio	<i>Saccharomyces exiguus</i> " <i>mandshuricus</i> " <i>marianus</i>  <i>Saccharomyces</i> sp.	<i>Mucor</i> sp. <i>Penicillium</i> sp. <i>Rhizopus</i> sp.  <i>Aspergillus</i> sp. <i>Penicillium</i> sp.
Figs	50 approx.	<i>Bacillus mycoides</i> Flügge <i>Flavobacterium rignensis</i> Bergey et al. " <i>tremelloides</i> (Schottelius) Bergey et al. <i>Serratia marcescens</i> Bizio	<i>Saccharomyces</i> sp.	<i>Aspergillus</i> sp. <i>Penicillium</i> sp.
Loganberries	200	<i>Bacillus mycoides</i> Flügge	<i>Saccharomyces mandshuricus</i> <i>Torula</i> sp.	<i>Penicillium</i> sp.
Raspberries	700	<i>Bacillus albus</i> (Sack) Bergey et al. " <i>mycoides</i> Flügge " <i>sitaticus</i> Neide <i>Pseudomonas syncyanca</i> (Ehrenberg) Migula	<i>Saccharomyces exiguus</i>	<i>Aspergillus</i> sp. <i>Mucor</i> sp. <i>Penicillium</i> sp.
Red currants	60	<i>Bacillus albolactis</i> (Löffler) Migula " <i>mycoides</i> Flügge " <i>sitaticus</i> Neide <i>Flavobacterium</i> sp. <i>Spirillum</i> sp.	<i>Saccharomyces</i> sp.	<i>Aspergillus</i> sp. <i>Penicillium</i> sp. <i>Rhizopus</i> sp.

## EXPERIMENTAL DATA ON FROZEN-PACK VEGETABLES

In general it may be said that frozen vegetables present a greater problem as far as microbial growth is concerned than do frozen fruits. Vegetable materials furnish food admirably suited to bacterial growth and, since not only the spoilage of the material but the healthfulness of the product as food is involved, a study of the organisms concerned with frozen vegetable spoilage is particularly important. In this connection it may not be amiss to point out that various investigators who have considered the possibility of dangers to health from the use of frozen-pack products have indicated that, when properly prepared and handled, there is no danger to public health from their use.

The data presented here are from 8 kinds of frozen-pack vegetables from many widely separated localities.

Fresh vegetables, when washed and macerated, are known to have normally a very high microbial content, which often runs into several millions per gram of material, even for good-quality products. The defrosted vegetables showed great variation in the numbers of microorganisms per gram, but these numbers were found to be rather high in most of the samples studied. The counts varied from a few hundred to several millions per gram and were made up predominantly of bacteria, although yeasts and molds were well represented on nearly all plates.

Table 4 lists the frequently encountered forms of bacteria, yeasts, and molds that were found in frozen-pack vegetables. It will be noted that generic names are the only ones given in some instances. The reason for this is that these organisms did not seem to be similar to any of the species described in the manuals consulted and are being held for further consideration.

It will be noted in table 4 that various species of the *Lactobacilli* are reported from beans, peas, and corn. They have been reported by Berry (2) in peas held in storage for 26 months. The *Lactobacilli* seem to play an important part in the spoilage of defrosted beans, corn, peas, and perhaps of other vegetables. Yeasts also play an important rôle in the spoilage of defrosted frozen-pack vegetables, especially green beans, Lima beans, and spinach, which sometimes develop a greasy appearance as soon as they start to spoil. This greasiness, in some instances, has been found due to a species of *Torula*.

Beets, corn, and tomatoes are likely to show surface mold in a day or two after defrosting and later to ferment. Frozen-pack vegetables, whether blanched prior to freezing or packed unblanched, have a tendency to spoil quickly at room temperature and should be cooked before defrosting or immediately thereafter. The need for great care in preparing vegetables for frozen pack is very evident when the list of microorganisms appearing after

TABLE 4.—Microorganisms found in frozen-pack vegetables, sealed in tin cans and other containers and stored at 15° F.

Product	Number micro-organisms per gram	Bacteria	Yeasts	Molds
Green beans	50 to 7,000	<i>Bacillus mycoides</i> Flüge <i>Flatobacterium butyri</i> Bergey et al. <i>Lactobacillus</i> sp. <i>Pseudomonas fluorescens</i> Ford <i>Sarcina flava</i> de Bary " <i>psychrocarctica</i> (Rubentschick) Bergey et al. <i>Flatobacterium flavescens</i> (Pohl) <i>Bacillus mycoides</i> Flüge	<i>Torula</i> possibly <i>glutinus</i>	
Beets	68,000	<i>Achromobacter</i> sp. <i>Flatobacterium diffusum</i> (Frankland) Bergey et al. <i>Lactobacillus</i> sp. <i>Pseudomonas oralis</i> (Ravenel) Chester	<i>Saccharomyces criguus</i> Kramir's Red <i>Torula</i>	<i>Penicillium</i> sp.
Corn	275-24,000	<i>Sarcina</i> sp. <i>Streptococcus</i> sp.	<i>Saccharomyces</i> sp. <i>Torula</i> sp.	<i>Rhizopus</i> sp.
Lima beans	300 to over a million	<i>Achromobacter</i> sp. <i>Bacillus coli</i> -like organism (unable to identify) " <i>fluorescens</i> Ford " <i>mycoides</i> Flüge <i>Flatobacterium suareolens</i> Soppeland <i>Sarcina hamaguchiae</i> Saito		<i>Cladosporium</i> sp. <i>Dematium</i> sp. <i>Monilia</i> sp. <i>Penicillium</i> sp.
Mushrooms	100-36,000			<i>Aspergillus</i> sp. <i>Mucor</i> sp. <i>Penicillium</i> sp. <i>Trichoderma</i> sp.



TABLE 4.—Continued

Product	Number micro-organisms per gram	Bacteria	Yeasts	Molds
Peas	7,000 to over a million	<i>Achromobacter guttatum</i> (Zimmermann) <i>Bergey et al.</i> <i>Acrobacter aerogenes</i> (Krusse) Beijerinck <i>Bacillus fusiformis</i> Gottheil " <i>mycoides</i> Flügge <i>Lactobacillus</i> sp. <i>Pseudomonas aeruginosa</i> (Schröter) Migula <i>Sarcina</i> sp.	<i>Saccharomyces</i> sp. <i>Torula</i> sp.	<i>Aspergillus</i> sp. <i>Penicillium</i> sp.
Tomatoes	20-50	<i>Sarcina lactea</i> Bergey <i>et al.</i>	<i>Saccharomyces mali</i> <i>Torula</i> sp.	<i>Rhizopus</i> sp.
Spinach	57,000 to over a million	<i>Achromobacter pellucidum</i> Harrison <i>Bacillus mycoides</i> Flügge <i>Flavobacterium aureum</i> (Ravenel) Bergey <i>et al.</i> <i>Sarcina</i> sp. <i>Streptococcus</i> sp.		

a year's storage at freezing temperatures is studied. The resemblance of some of the bacteria in frozen-pack products to pathogenic types suggests that great care should be taken to so label frozen-pack vegetables that they will not be mistaken for a cooked or canned product and eaten directly from the package.

#### SUMMARY

This paper presents the results of an investigation, extending over a period of 5 years, into the microbiology of approximately 3,000 samples of frozen-pack fruits and vegetables contained in barrels, hermetically sealed tin cans, glass jars, and paper containers, derived from the principal production centers of the United States, which may be summarized as follows:

The fresh strawberries from eastern, southern, and northwestern sources, after hulling and washing, showed a microbial content varying between the limits of a few thousands and a million or more organisms per gram of material, the content varying with the efficiency of washing, the degree of ripeness, and the soundness of the fruit.

The analysis of the frozen-pack product from the same sources after one year's storage at 15° F. in sealed tin cans and paper containers showed an average reduction in microbial content of 99.3 per cent.

No microorganisms pathogenic to human beings were isolated from the frozen-pack product, but several species of bacteria from animal or human sources were found in the fresh strawberries, thus emphasizing the need for strict sanitary control of the packing operations.

The microorganisms surviving the frozen-storage treatment in strawberries in sealed tin cans and paper containers for a year included 7 genera of fungi, a genus of yeast, and 5 genera of bacteria, the latter including approximately 30 species.

Numerous species of bacteria, and several species of molds and yeasts survived 3 years' storage at 15° F. in sealed tin cans of frozen strawberries.

Examination of other frozen small fruits including blackberries, cherries, figs, loganberries, raspberries, and red currants after storage for a year in sealed tin cans and other containers at 15° F. showed the presence of molds, yeasts, and bacteria, the numbers ranging between 50 and 2250 organisms per gram. These microorganisms were found to belong to 4 genera of molds, 2 of yeasts, and 6 genera of bacteria including about a dozen species.

Bacteria of animal or human origin were isolated from frozen-pack figs.

Microbiological examination of frozen-pack beans, beets, corn, Lima beans, mushrooms, peas, tomatoes, and spinach after storage for a year in sealed tin cans and paper containers at 15° F. showed numbers of viable organisms ranging from 20 to over a million per gram of material including

7 genera of bacteria, 2 of yeasts and 8 of molds, the higher counts being obtained from frozen products having an unknown storage history.

#### CONCLUSIONS

Microorganisms representing the molds, yeasts, and bacteria in considerable numbers are able to survive frozen storage in sealed tin cans and other containers of fruits and vegetables held at 15° F. for periods of 1 to 3 years, at least.

The occurrence of undesirable organisms of human or animal origin, both in the fresh material and in some of the frozen-pack products, emphasizes the need for careful sanitary control during packing operations.

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# INCREASED EFFICIENCY OF CHLOROPICRIN FOR NEMATODE CONTROL WITH BETTER CONFINEMENT OF THE GAS<sup>1</sup>

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During the years 1930 to 1932 the nematology division of the Station conducted a series of small-scale experiments with chloropicrin for the control of the common root-knot nematode, *Heterodera marioni* (Cornu) Goodey. With the gradual improvement in technique of application, as pointed out in this paper, constantly better results were obtained. The culmination of the series in the greenhouse was a test that demonstrated the value of adequate confinement of the gas in increasing the efficiency of the treatment. The development of methods for gas confinement is presented in another paper (3). Accounts of field-plot experiments for nematode control are likewise presented elsewhere (4, 9).

## CHLOROPICRIN AS A FUMIGANT

Chloropicrin,  $\text{CCl}_3\text{NO}_2$ , has been called a "tear gas" because of its high efficiency as a lachrymator, but it is completely different from the true tear gas (chloroacetophenone,  $\text{C}_6\text{H}_5\text{COCH}_2\text{Cl}$ ), used as such generally at the present time. Pure chloropicrin is a colorless liquid, though the commercial preparation is yellowish. It is considerably heavier than water, with a specific gravity of about 1.64 at ordinary temperatures. It has an oily appearance and a sweetish, not unpleasant odor. Its vapor tension is about the same as that of water. The gas has an immediate irritating effect on the eyes, and in strong doses is irritating to the lungs and causes nausea and vomiting. Contact with the skin causes a burning that may become serious unless remedies are applied. The harmful effects of the gas in low concentrations are only temporary. High concentrations are dangerous. When these facts as to inherent possibilities of injury are thoroughly understood and ordinary precautions are taken, the chemical can be used safely by the ordinary operator, indeed much more so than can the deadly poison hydrocyanic acid gas or the highly explosive carbon bisulphide. The toxicity of chloropicrin to the lower forms of animal life, particularly to insects, is well established in the literature, most of which, up to 1930, is referred to by Roark (10) and Gersdorff (2), whose very complete bibliographies cover also the fields of the chemistry and physics of the substance and its toxicity to higher animals, including man.

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## EXPERIMENTATION

The experiments described in this paper were directed primarily toward the control of the root-knot nematode. They include (1) miscellaneous preliminary laboratory tests, to establish toxicity to nematodes; (2) a series of greenhouse tests, incorporating gradually improved technique of application; and (3) small plot tests in the field.

## LABORATORY TESTS

*Test 1.*—*Heterodera marioni* eggs in egg masses were killed by exposure to chloropicrin gas of unknown (but rather high) concentration in closed dishes in the laboratory during an over-night exposure of about 18 hours. Killing was proved by attempts to hatch them by methods described in another paper (8). No hatching whatever occurred, whereas control eggs hatched freely. The determination of lethal concentrations and periods of exposure remains for further experimentation.

*Test 2. Studies on the Toxicity of Chloropicrin in Solution.*—Chloropicrin is almost completely insoluble in water (solubility 0.14 per cent). Small drops of the chemical were placed in Syracuse dishes of water, where they rested on the bottom as distinct droplets. *Heterodera marioni* larvae were inserted. Even when they were immediately adjacent to the droplets of the chemical they remained alive and active for several hours. Ten per cent solutions of chloropicrin in 95 per cent alcohol were made up, and these diluted with water in various proportions, with a resulting faintly whitish homogeneous emulsion. Tests with these showed almost instantaneous killing of larvae at all concentrations down to 1 part of the alcoholic solution to 100 parts of water that contained 0.1 per cent chloropicrin and 0.9 per cent alcohol. This concentration of alcohol by itself is not at all lethal to nematodes. Dilutions to just half this concentration (.05 per cent chloropicrin) were not lethal, or at least not quickly so.

## GREENHOUSE EXPERIMENTS

Following the preliminary laboratory tests, a number of greenhouse experiments were conducted in which the following standard technique was employed:

Heavily nematode-infested soil, was selected either directly from known badly infested fields, or from good top-soil areas, and then heavily inoculated with infested roots. The soil was next thoroughly mixed by repeated shovelings and placed in the containers in which the tests were to be conducted. The chemical was inserted, as described for each experiment. An adequate number of nontreated controls was always left for comparison. The indicator-crop method described elsewhere (5) was the basis of comparison.

*Test 3.*—This test, with quantities of chloropicrin ranging from 250 to 2,000 pounds per acre, was almost a complete failure for reasons not thoroughly understood but probably related to the inclusion in the soil of undecayed infested roots and to the too rapid escape of the gas. The heaviest application showed only about 60 per cent control and the others less than that.

*Test 4.*—In this small-scale test, wooden tubs of .65 cubic foot capacity were used as containers. Chloropicrin, at the rate of 4 cubic centimeters per tub (approximately 1000 pounds per acre), was inserted into the center of each of 4 tubs, 2 of which were covered immediately with circular pieces of tar-impregnated mulching paper, and 2 left uncovered. Seven tubs of the same soil were available as controls, for comparison. Results from this test in terms of gall counts on indicator plants, taken about 30 days after planting, were as follows:

Control tubs, gall count, average of 7,  $1593 \pm 69$ .

Chloropicrin treated, covered, (1st 40, 2nd 35); av. 37.5.

Chloropicrin treated, not covered, (1st 70, 2nd 500); av. 285.

The covered tubs showed an average of 97.7 per cent control. The uncovered varied widely, but obviously were not nearly so successful in nematode reduction as the covered. Here is an indication, then, that covering the soil makes for better confinement of the gas and consequent better control of the nematodes. This point was followed up intensively in later experiments.

*Test 5. An Experiment with Coated Papers Designed to Hold the Chemical in the Soil.*—The set-up of this experiment was similar to the foregoing, except that the containers were glazed stone jars of 4-gallon (15 liter) capacity. Rates of application of chloropicrin were based upon the volume of soil treated as compared with that of an acre-foot of soil. In actual practice in the pineapple field, just half the rate specified (Table 1) per acre would be used to get the same concentration of gas in the soil actually treated, in view of the fact that only alternate 3-foot strips are treated and planted. For gas confinement, the jars were covered with Kraft paper coated with a thin layer of waterglass (sodium silicate), previously applied and permitted to dry. The treated paper was cut to size and pasted tightly in place over the jar with ordinary starch paste, the edges being further sealed with an additional application of water glass. Certain jars had ordinary mulching paper, instead of the treated paper, glued over the tops as seals.

The chemical was inserted through a small hole in the cover by means of a pipette, to a depth of about 3 inches, after which the hole was immediately sealed with a sticker, which was covered finally with waterglass.

Because of the tendency of this water-glass sizing to crack upon exposure

to alternations of high and low temperatures or of degrees of dryness of the atmosphere, an effort was made to prevent it by coating the paper with self-annealing rubber latex. This proved inadequate as was shown by the gradual development, from the second day, of a fine network of crackings. The laboratory tests heretofore referred to (3) showed that when such cracks appeared the efficiency of the sizing for holding gases was immediately greatly reduced. That such occurred in this case was shown by the fact that on the fifth day after application of the chemical, when covers were removed, the odor of the chloropicrin had entirely disappeared from all jars except two of the highest applications, which were especially well sealed.

All the treatments were in triplicate, with 6 nontreated controls, 3 with covers and 3 without. The rates of application and results in nematode control are given in table 1.

TABLE 1.—*The results in nematode control of applications of chloropicrin in 4-gallon stone jars sealed with water-glass-coated paper and tar-impregnated mulching paper<sup>a</sup>*

Pot number	Rate of application		Nematode gall-count		Reduction
	Per jar	Per acre	3 jars	Average	
	<i>g.</i>	<i>lbs.</i>			<i>per cent</i>
1 to 3	.625	250	52, 32, 142	75.3	70
4 to 6	.750	300	22, 25, 5	17.3	93.1
7 to 9	.875	350	0, 23, 10	11.0	95.6
10 to 12	1.00	400	0, 20, 87	35.7	86.0
13 to 15	.875	350	18, 35, 263	105.3	58.0
16 to 18	1.00	400	14, 52, 46	37.3	85.1
19 to 21	0 (covered)	0	156, 113, 492	254	.....
22 to 24	0 (not cov.)	0	274, 69, 119	154	.....
40 to 42	1.50	600	40, 9, 4	17.7	93
Averaging jars 41 and 42 in which odor of gas was still present after 5 days					9.4

<sup>a</sup> Jars 13 to 18 were covered with mulching paper instead of water-glass-coated paper.

In considering the nematode results in this table it should be noted that the covers were not highly efficient for their main purpose, that of confining the fumigant. In spite of this weakness, fairly good efficiency in nematode control is manifest, particularly when results are compared with previous tests in which covers were not used. An apparent increase in efficiency with increased rate of application is evident in the first 3 series. In the

fourth, unfortunately, the cover of the last jar, being loose allowed rapid escape of the gas. This resulted in a less efficient killing of nematodes and a consequent increase in the average gall count of the three. In 1 jar each of the third and fourth series, 100 per cent efficiency was apparently attained. In jars 13 to 18, with high applications, but with mulching paper covers, rather low average efficiency occurred. In the final jars of the entire series, in which efficient confinement of the gas was manifest by odor of chloropicrin after 5 days, 97.4 per cent control was obtained. In the light of further intensive studies on soil fumigation, it would appear that some of the survival that occurred throughout was due to nematodes present within sound roots.

Another feature of this test, not disclosed in the table, involved the introduction, before treatment, of *Heterodera marioni* egg masses and infested pineapple roots into 2 of the jars (Nos. 40 and 19) 1 treatment and 1 control. The eggs were contained within small glass cells covered with silk bolting cloth, which, attached to strings, were thrust to a point well below the surface, as were also the infested roots. At the time of removing covers these were removed and egg masses from both sources (cells and roots) were placed in drops of water on slides for observations on viability. The free eggs from the control jar showed a high percentage of hatching; those from the chloropicrin jar showed none at all. Complete efficiency was thus attained in killing the organism in free egg masses. Eggs from the exposed roots all showed a high percentage of hatching, indicating lack of efficient penetration of the gas into the interior of such roots. As suggested before, the presence of such undecayed roots undoubtedly accounts for at least a part of the survival of infestation in the treated jars. On the whole, the better nematode control in this test, as compared with that of previous tests, would appear to demonstrate the value of a gas-tight cover for the purpose of prolonging the exposure of the organisms to the fumigant.

*Test 6. Chloropicrin treatments in containers sealed with glue-coated papers.*—For this test, an effort was made to improve over the preceding one by the use of covers of glue-coated paper, a material that does not crack upon exposure. The glue used was a water-proof aircraft adhesive prepared according to U. S. Government formula No. 11 (11), which, in laboratory tests, had shown high efficiency in preventing the escape of chloropicrin. The series consisted of the usual heavily infested soil in 5-gallon (20 liter) iron paste buckets. Chloropicrin was inserted in alcoholic solution of such concentration that multiples of 2.5 cubic centimeters gave the desired magnitudes of application in the different jars of the series. Four different rates of application were used,—200, 250, 300, and 350 pounds per acre on the basis of soil volume as related to the acre foot.



As applied in the field, in alternate strips, just half these quantities would be used per acre. Length of exposure to the fumigant was an important feature of this test, the gas-confining covers being removed in triplicate lots for each rate at 2, 4, and 7 days after treatment.

When covers were removed at the ends of the specified periods, very high efficiency in chloropicrin retention was noted in general. The gas was exceedingly strong in the 2-day jars, only a trifle less so in the 4-day, and still quite evident in the 7-day containers. Results in nematode control are shown in table 2.

TABLE 2.—*Results of soil treatment with chloropicrin in containers covered with highly impervious glue-coated papers*

Pot number	Application per acre, pounds	Period confined under cover days	Nematode gall count Containers				Percentage reduction based on controls
			1st	2d	3d	Average	
1 to 3	200	2	43	89	96	76.0	86.5
4 to 6	200	4	18	61	16	32.0	94.3
7 to 9	200	7	0	6	49	27.0	95.2
10 to 12	250	2	56	36	486	193.0	65.6
11 to 15	250	4	155	59	227	147.0	73.8
16 to 18	250	7	17	24	4	15.0	97.3
19 to 21	300	2	5	1	10	5.3	99.1
22 to 24	300	4	0	0	12	4.0	99.3
25 to 27	300	7	0	3	1	1.3	99.8
28 to 30	350	2	15	4	0	6.3	99.0
31 to 33	350	4	2	4	7	4.3	99.2
34 to 36	350	7	0	0	1	0.3	99.95
37 to 41	None	7	678, 971, 238, 300, 620				561.0
							.....

Certain irregularities and inconsistencies appear that can be explained only by irregularities in gas confinement by the covers of different jars, irregularities in root growth of indicator plants, or by the presence in some jars of large, sound roots containing protected infestation. Probably all three conditions are factors. Roots of control plants were badly rotted and gall count on them was definitely lower than would have appeared had such decay not occurred. Percentage control figures, therefore, should be higher throughout than those recorded. Statistical analysis of results from 3 pots only is hardly justified. Even without such analysis, definite trends in the results are apparent. The consistent increase in efficiency of treatments with increase in period of exposure to the confined gas is evident throughout. With the higher rates of application, 300 and 350 pounds per acre, (150 and 175 pounds as applied in the alternate-row system of treatment)

100 per cent control occurred in 6 cases out of 18 and better than 99 per cent in the balance.

#### SMALL FIELD-PLOT TESTS

Small field-plot tests were conducted to obtain preliminary information on the practicability of the use of soil covers, and the benefits to be derived thereby in improved nematode control. Several exploratory tests are not reported, inasmuch as they served their purpose only to the extent of teaching lessons in technique applied in later trials. They did serve to show that chloropicrin is not likely to prove highly effective as a soil fungicide in saturated soil. They likewise indicated the value of a solid frame surrounding the plot sunk to a depth of several inches, with the chemical-imperious cover sealed in place upon it thus eliminating rapid diffusion of the gas away from the soil. And, finally, verification of the results of test 6, heretofore described, were obtained in part, from the fact that 4-day confinement of the gas under a cover gave better control than 2-day, and 2-day confinement better than any shorter period, or no cover at all.

*Test 7. A Plot Test with Glue-coated Covers Sealed around the Edges.* This test was installed in 4 × 8 ft. galvanized-iron-enclosed, isolated plots, like those described by Byars (1) and those used by Godfrey and Hagan (7) in trap-crop studies. The test involved an effort to procure good confinement of the gas in the soil, as was done in test 6 in the greenhouse, by gluing the edges of the paper to the sides of the plot frame. The attempt was not highly successful, for in all cases the seal became loose at 1 or more points by the second day. When covers were removed in 4 days, the odor of chloropicrin did not persist. Rates of application were 400 and 300 pounds per acre. Each treated plot was paired with an adjacent plot as control, the soils in the 2 having been thoroughly mixed in advance, to obtain uniformity of infestation. Different pairs of plots varied widely in infestation, as shown by indicator-crop readings in the controls. After treating and subsequent aeration, cowpea seeds and tomato plants were planted in alternate rows as indicator plants. The tomatoes, becoming more quickly established, had much larger and deeper root systems than cowpeas, a fact reflected in higher gall counts per root system. For soil sterilization tests tomatoes proved much superior as indicator plants, probably because of the reduction of beneficial nodule bacteria with resulting detriment to cowpea growth. Detailed results in gall counts and in effects on plant growth, are given in table 3.

Detailed consideration of these results is necessary to their proper interpretation. Two lots, number 1 and 2, were heavily infested, as shown by the gall counts in the controls on both tomatoes and cowpeas, whereas the other 2 lots were only lightly infested. In the former, the treatment

TABLE 3.—Results from test 7, chloropicrin with covers sealed in place, in root-gall count and effect in plant growth. (N, nontreated; Tr. treated)

Indicator	Rate per A. pounds	Number of plants		Gall count				Top weight of plants			
				N	Tr.	Difference	D/PE	Per cent reduction	N	Tr.	Difference
		N	Tr.								
Tomatoes											
Lot											
1	400	8	8	305 ± 28.6	.75 ± .26	- 304 ± 28.6	10.6	99.8	2.3 ± .31	5.1 ± .6	2.8 ± .68
2	400	8	8	607 ± 90.0	.94 ± .36	- 513 ± 96	5.3	85	6.0 ± 1.4	12.1 ± 2.0	6.1 ± 1.2
3	400	9	6	11.8 ± 2.8	3.6 ± 1.9	- 8.2 ± 3.4	2.42	70	33.9 ± 2.8	26.3 ± 3.8	-7.6 ± 4.7
4	300	7	7	60.0 ± 12.9	.28 ± .18	- 59.7 ± 13	4.63	99.5	22.6 ± 2.8	13.8 ± 2.4	-8.8 ± 3.7
Cowpeas											
Lot											
1	400	45	24	67 ± 5.7	2.8 ± .57	-64.2 ± 5.7	11.2	95.8	Size differences in cowpeas, irregular and not significant.		
2	400	35	34	126 ± 22.8	6.4 ± 1.5	- 120 ± 23	5.2	95.1			
3	400	40	47	15 ± 1.7	.34 ± .07	-14.66 ± 1.7	8.6	97.8			
4	300	40	36	31 ± 16.2	.64 ± .18	-30.36 ± 16.2	1.9	97.9			

brought about striking reduction in nematodes, and this was reflected in significant increase in tomato plant growth, even in the short period of 30 days. With the cowpeas, while comparable reduction in nematode infection occurred, corresponding increase in plant growth did not occur, probably because of an evident simultaneous killing of the beneficial legume bacteria. In the low infestation lots the nematodes were not sufficient in numbers to retard plant growth materially, even in the controls; so such reduction as occurred from the treatments did not manifest itself in the growth of the plants.

Examination of indicators, plant by plant, disclosed somewhat erratic nematode control, indicating lack of satisfactorily uniform distribution of the chemical through the soil. In lot 2, for example, 2 tomato plants near the end of the plot showed 246 and 443 galls, respectively, the other 6 averaging only 10. These 2 plants, then, are responsible for the rather high average that had to be recorded for the treatment. It is very possible that the unreliable applicator failed to inject into the region in which they were set. Without the 2, that is, for the remainder of the plot, control was 98.4 per cent. The same irregularity was manifest in the cowpeas in the same region, 3 plants showing a high gall count, the other 31 plants a very low one. A further contributing factor to lack of high efficiency in control was undoubtedly the failure of the seal after a few hours.

*Test 8. Chloropicrin in a Home-garden Plot Confined with a Gas-imperious Paper Cover Sealed to a Board Frame Border.*—A final experiment in this series of small plot tests was conducted in a home-garden plot heavily infested with nematodes. Flowers of various kinds, including particularly carnations, *Dianthus caryophyllus* L., and *Calendula officinalis* L. were failing completely. The ground selected for treatment comprised an area of approximately 10 by 20 feet, inclosed within a 12-inch board border sunk to a depth of 10 inches. The soil was in fine tilth and fertile, having received a recent addition of well-rotted stable manure. A parallel area of equal size was reserved for nontreated control plot. Chloropicrin was applied at a heavy rate—800 pounds per acre—in holes about 6 inches deep, spaced 12 inches apart. The plot was immediately covered with a large sheet of glue-coated paper, water-proofed with a coating of tar paint, which was glued to the frame border. The cover was left on for 5 days. Unfortunately, at some time during this period the paper became broken, with a hole about the size of a man's foot. When the cover was removed the odor of chloropicrin was no longer apparent. The desired efficiency in gas confinement was not fully attained. An immediate effect of the treatment, however, was evident in the finding of many dead insects, particularly burrowing cockroaches (*Pycnoscelus surinamensis* L.) on the surface of the soil.

The plot was left open a day for aeration, then tomatoes and cowpeas were planted, as nematode indicators, in alternate rows at one end of the plot. Within 3 weeks after planting striking differences in favor of the treatment were evident in tomato-plant growth. At the end of 30 days alternate plants were removed for observation on root infection and for quantitative determination of differences in plant growth (Fig. 1). The results are summarized in table 4.

The outstanding result of this test was the striking increase in top weight of the tomato plants, nearly 7-fold, brought about by the treatment, after only 1 month of growth. The condition of the plants indicated that this would have been followed by equally striking yield differences, but the test was not followed through to that point. Root-gall-count differences, while significant, were not so great, on the percentage reduction basis, as in pot studies herein reported; but the lack of high percentage reduction was again brought about by a few individual plants with high gall count in the treated plot (2 of them tomatoes with 520 and 484 galls, respectively), where pockets of soil escaped the benefit of the treatment. Such



FIG. 1. Tomato plants showing the beneficial effect of a heavy soil treatment with chloropicrin, as described under Test 8 in the text. The average top weight of 11 plants from the treated plot after 30 days' growth was 7 times that of the average from the nontreated. Scale in inches.

TABLE 4.—Results from a nematode-control experiment with chloropicrin in a small home-garden plot in Honolulu, Hawaii

Indicator plant	Number of plants		Gall count					Top weight, grams				
	N	Tr	N	Tr	Diff.	D/PE	Percent-age reduction	N	Tr	Diff.	D/PE	Percent-age increase
Tomato	8	11	665.3 ± 31.2	134.7 ± 38.9	531.0 ± 50.0	10.6	80.0	20.0 ± 3.2	139.1 ± 11.3	119.1 ± 11.7	10.2	595
Cowpea	31	36	118.9 ± 12.4	13.1 ± 2.0	105.8 ± 12.5	8.5	89					

pockets either contained sound roots of the previous infested crop or were missed by the applicator. Similar escape of some of the soil under cowpea plants was also evident, 1 plant showing 116 galls and another 46, as compared with the average over all of only 13. It is believed that improved technique of application will overcome a large part of this escape from the treatment, in pockets.



FIG. 2. A secondary benefit from chloropicrin fumigation of the soil lies in its effectiveness in weed killing. Two plants of nutgrass, *Cyperus rotundus*, with the tubers cut to show the interior. Left, normal; right, completely killed, with the tuber soft and brown throughout. About  $\times \frac{1}{4}$ .

## EFFECT OF CHLOROPICRIN ON WEEDS AND GROWING PLANTS

Another benefit from the chloropicrin treatment in this experiment, which is worthy of record, was its high efficiency in killing nut grass, *Cyperus rotundus* L., a serious weed in the Hawaiian Islands and one that is very difficult to eradicate (Fig. 2). Many plants that were growing at the time of treatment were killed outright. Examination of underground "nuts" or corms showed them to be brown throughout and decaying. After about a month a few plants appeared at the surface; these were always traceable to corms a foot or more deep in the soil. Some at a foot depth were partially discolored interiorly, but had sufficient life to bring about growth and thus to reestablish the weed in the soil. In the treated soil, however, there developed only a small fraction of the number of plants that continued to develop in the nontreated soil.

As additional evidence of the lethal effects of chloropicrin on growing plants, the results of applications to growing pineapple plants are briefly reported. In a small block of 50 plants about 6 months old, chloropicrin was inserted into the soil at the base of each plant with the Vermorel injector, at the rate of 2 cubic centimeters (3.3 grams) per plant. Within a few days the plants showed signs of injury and at the end of a month all but one were dead. Since the pineapple plant is ordinarily very hardy to unfavorable environmental conditions, this is a striking demonstration of the lethal effects of the gas on growing plants.

Another paper (6) describes the effects of low concentrations of the gas in the atmosphere on plants in the greenhouse, and emphasizes the necessity for precautions in applying treatments in enclosed spaces, such as obtain in greenhouses. It is completely impracticable to conduct a greenhouse soil fumigation with growing plants elsewhere in the same house.

## DISCUSSION

Some of the greenhouse tests reported in this paper demonstrate the possibility of obtaining under ideal conditions complete control of the root-knot nematode by means of chloropicrin fumigation. None of the small plot tests was so successful. Lessons were learned from the series, however, that indicate the possibility of a high degree of efficiency when the best possible technique of application and of soil management is followed. The highest efficiency is obtainable when the following conditions are fulfilled:

- (1) All roots of a previous infected crop that are left in the soil must be well rotted in order to allow efficient penetration of the gas to the points where the nematode larvae and eggs are imbedded. This requires a period of from 2 to 4 weeks or more, after the removal of the old infested crop,



under moisture, temperature, and soil-nutrient conditions favorable for decay of organic matter.

(2) The soil at the time of treatment should be in a good tilth, free from clods, and not excessively moist. (The exact moisture content that is unsafe is not known beyond the fact that treatments applied to soils that were temporarily above the "moisture equivalent" point always showed low efficiency. More precise studies in this connection are desirable.)

(3) Rates of application of 300, 350, and 400 pounds per acre (approximately 15/16 of the same figures in kilograms per hectare) have all shown good results, with efficiency in nematode control in proportion to the quantities used. The economic law of diminishing returns applies to the use of chloropicrin as it does to the use of fertilizers and other materials whose function is to increase crop yields.

(4) Points of application of chloropicrin should be not greater than 18 inches (46 centimeters) apart, and should be from 6 to 8 inches deep.

(5) The gas must be confined in the soil as efficiently as possible by means of a cover of some material that is highly impervious to it, and this cover must leave no openings that permit the free escape of gas to the open air. The more efficient the cover, the better the results are likely to be, all other conditions being equal. The cover should be maintained for at least 2 days, and preferably for 4 or more. After removal of the cover a delay of a day or 2 before planting is desirable to permit the complete escape of the free gas from the soil.

#### SUMMARY

Laboratory, greenhouse, and small plot tests with chloropicrin for nematode (*Heterodera marioni*) control were conducted in Hawaii during the years 1930 to 1932. In the greenhouse tests constantly improved results were obtained with improved technique of application. Adequate confinement of the gas in the soil with a cover highly impervious to the gas was found to be indispensable to best results. Ordinary Kraft paper, coated with a thin sizing of animal-product glue or casein glue, when placed over the soil and sealed at the edges, proved to be highly efficient for the purpose. Chloropicrin, applied at rates of 300 and 350 pounds per acre in closed containers, with covers as described, gave better than 99 per cent control, with 100 per cent control in several cases. The same technique applied to small field plots, with the covers sealed to metal or wood frames about the plots, did not show quite so high percentage control of nematodes but did result in remarkably increased vigor of tomato plant growth. In the treated portion of one garden plot, these plants, after only 30 days' growth, were 7 times as heavy in green top weight as the controls. Although good nematode control was manifested with cowpea-indicator plants

in the same experiment, improved plant growth did not occur because of the lethal effects of the treatment on the beneficial legume-nodule bacteria. Conditions essential to the high efficiency of chloropicrin fumigation for nematode control are (1) complete decay, before treatment, of any residue in the soil of infested roots of a previous crop; (2) good loose tilth and absence of excessive moisture; (3) rates of application of from 250 to 400 pounds per acre, depending upon efficiency desired and economic considerations and upon the efficiency of the measures for gas confinement; (4) points of application not more than 18 inches apart; (5) efficient confinement of the gas in the soil by means of a cover of gas-impervious material such as glue-coated paper, with the edges sealed to a frame or sunk well beneath the surface of the soil.

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# THE HONEYBEE IN RELATION TO THE OVERWINTERING AND PRIMARY SPREAD OF THE FIRE-BLIGHT ORGANISM

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The elimination of the source of the inoculum is an essential factor in fire-blight control. A number of investigators have demonstrated that the oozing hold-over canker is an important source of primary inoculum (2, 9, 3), and others. Most control measures have been based upon this observation. An additional source of primary inoculum was mentioned by Rosen (5) who stated "that the fire blight germ was isolated time and again from this material and from the bees themselves taken from the hives in early spring prior to any signs of blight in the orchard." "This material" refers to honey plus comb, pollen cells, and brood cells taken from the hives, throughout the summer, winter, and early spring prior to the development of blight. He concludes, "Thus it was proved that the infected beehive is at least in part responsible for the current season's blight. . . ." The importance of this statement to both beekeepers and fruit growers gave rise to the investigation of this phase of the fire-blight problem under Ohio conditions.<sup>1</sup>

On April 22, 1932, 2 3-frame nucleus hives, containing brood, honey, and enough bees to care for the brood were assembled at the university apiary at Columbus, Ohio. On the same day several flasks of bouillon were inoculated by pouring into them small amounts of bouillon cultures of 2 virulent isolations of *Bacillus amylovorus* (Burr.) Trev. The following day these hives and flasks of inoculum were transported to the Flagg orchards in Scioto County, Ohio, and hive No. 1 was infested with the bouillon culture of the fire-blight organism. This inoculum was smeared over the entire contents of the hive so that the combs, frames, hive body, and bees were covered and some of the culture was poured into the hive body.

On April 25 this infested hive was placed beneath a Yellow Transparent apple tree (Tree No. 1), which had been enclosed in a bee-tight cheesecloth cage, and the bees released. The blossoms were just beginning to open. A check was prepared by tying a large cheesecloth bag securely over several branches of this tree.

On the following day hive No. 2, which had not been infested, was placed

<sup>1</sup> The writers wish to express their gratitude to the following fruit growers for their cooperation in this work: Mr. C. E. Drumbheller manager of the Flagg orchards, Mr. Blair Adams, and Mr. Herman Mantle, and to Dr. W. E. Dunham, of the Ohio State University, who kindly provided the honey bees and apiary equipment used in these investigations.

beneath a similarly caged tree of the same variety (Tree No. 2). Just before the bees were released in this second cage, nineteen of the lowermost blossom clusters were inoculated with an atomizer containing a portion of the bouillon culture used to infest hive No. 1. A check was prepared in this cage also as described for tree No. 1. These trees were in full bloom April 28 and 29, and there was no precipitation while the blossoms were open. Results were recorded on May 18.

On May 2 both hives were transported to the Adams fruit farm near Columbus, and the bees confined to the hives. On May 4 hive No. 1 was placed beneath tree No. 3, a cheesecloth-enclosed Grimes; hive No. 2 beneath tree No. 4 and the bees released. Full bloom occurred on May 5 and the bees were removed on May 8. When hive No. 2 was removed a handful of bees, which escaped through a small rent in the top of the cage, were clustered on the outside. Results were recorded on May 24.

An experiment similar to the one at the Flagg orchards was conducted at the Mantle and Mantle fruit farm near Painesville, in northern Ohio. Here one large "V"-shape Yellow Transparent tree was enclosed by a cheesecloth cage. A partition of the same material was placed through the center of the tree, thus dividing the cage into 2 sections with a part of the same tree in each end of the cage.

On May 13, a hive of bees was placed in each end of the cage and the bees released. On May 15, the hive in one end of the cage was infested with a virulent bouillon culture of 2 isolations of the fire-blight organism in the manner described previously for the infestation of hive No. 1 at the Flagg orchards. On the same day, a portion of this culture was atomized into 25 of the lowermost blossom clusters in the opposite end of the cage. The tree was in full bloom on May 18 and the results recorded were obtained June 1.

The data of the 1932 experiments are presented in table 1. It will be observed that no blight developed on trees 1 and 3, and we may assume that no blight bacteria were carried by the bees from the infested hives into the open blossom clusters either in the Flagg orchards or in the Adams orchard.

In tree No. 2, on which 19 blossom clusters were inoculated with blight bacteria before the bees were released inside the cage, 131 additional blossom clusters, or 66.9 per cent of the total number visited by bees developed blight. Blossom clusters on this tree protected from bee visitation by cheesecloth bags remained healthy. The results of this cage experiment indicate that fire-blight bacteria were carried from one blossom to another by honey bees.

One blighted blossom cluster was found on tree No. 4 in the Adams orchard, and may have been inoculated with bacteria carried by the bees from hive No. 2 at the Flagg orchards. This explanation appears improbable when it is noted that only one cluster out of 1165 was blighted. The

TABLE 1.—Results of 1932 cage experiments conducted to determine relation of the honeybee to the overwintering and primary spread of *Bacillus amylovorus*

Tree No.	Location	Method of inoculation	Date bees placed in cage	Date of full bloom	Date bees removed	Date observations were taken	Healthy blossom clusters	Blighted blossom clusters	Blighted clusters per cent
1	Flagg orchards	Hive No. 1 placed in cage <sup>(1)</sup> Check <sup>(3)</sup>	April 25	April 28-29	May 2	May 18	494 29	0 0	0.0 0.0
2	Flagg orchards	Hive No. 2 placed in cage <sup>(2)</sup> Check <sup>(3)</sup>	April 26	April 28-29	May 2	May 18	74 16	150 0	66.9 0.0
3	Adams Fruit Farm	Hive No. 1 placed in cage <sup>(1)</sup>	May 4	May 5	May 8	May 24	745	0	0.0
4	Adams Fruit Farm	Hive No. 2 placed in	May 4	May 5	May 8	May 24	1165	1	0.085
5	Mantle orchard	Hive inoculated <sup>(4)</sup>	May 13	May 18	May 19	June 1	1364	8	0.583
5A	Mantle orchard	Tree inoculated <sup>(5)</sup>	May 13	May 18	May 19	June 1	750	790	51.3

(1) Hive No. 1 infested May 23 with bouillon culture of *B. amylovorus*.(2) Tree No. 2 inoculated May 26 by atomizing 19 of the lowermost blossom clusters with a virulent bouillon culture of *B. amylovorus*.

(3) Checks protected from bee visitation by cheesecloth bags.

(4) Hive was infested May 15 with a bouillon culture of *B. amylovorus*.(5) Tree 5A was inoculated May 15 by applying a virulent bouillon culture of *B. amylovorus* to 25 of the lowermost blossom clusters with an atomizer.

location of this cluster near the top of the cage and near a small rent in the cheesecloth suggests that a bee returning to the cage after visiting an adjacent Kieffer pear tree severely infected with blossom and twig blight might have inoculated this cluster. A few bees did escape through this rent and were clustered on the outside of the cage when the hive was removed. Or, meteoric water might have inoculated this cluster by splashing bacteria from the adjacent pear tree.

In the Mantle orchard, 1364 blossom clusters on the side of the tree enclosed in the cage with the infested beehive remained healthy. Eight clusters on this side of the tree, or about 0.6 per cent of the total, were blighted. The bees may have carried the blight organism from the infested hive to the open blossom clusters, but the small amount of blight and the location of the blighted clusters near the partition suggest the possibility of inoculation by splashing rain drops carrying the blight organism. A heavy rain occurred during anthesis. Gossard and Walton (1) describe an experiment in which it appeared that the blight organism was transferred through cheesecloth by splashing rain. Miller (3) also states that splashing meteoric water is one of the most important agents concerned in the spread of fire-blight inoculum in Wisconsin. Tullis (9) draws the same conclusions for Michigan.

The spread of the blight organism to more than half of the blossom clusters on the side of the tree containing the 25 inoculated blossom clusters again demonstrates that bees carry the bacteria from blossom to blossom.

From these data it appears that, while honey bees readily carried blight bacteria from infected blossoms to others that they visited, they rarely if ever carried the organism from the hive to the blossoms.

#### CAGE EXPERIMENTS IN 1933

On April 10, 1933, bouillon cultures were prepared from two isolations of *Bacillus amylovorus* used in the 1932 tests. Two days later this culture was applied by means of an atomizer to the frames, combs, honey, bees, and brood in a standard hive containing the combs used in hive No. 2 at the Flagg orchards in 1932. This treatment was repeated on the following day with a similar culture. Apple twigs inoculated with a portion of this culture developed characteristic symptoms of blight.

On April 18, 2 3-frame nucleus hives were assembled at the University. The first, designated as No. 1, consisted of 3 frames of brood, honey, and bees taken from the hive inoculated April 12 and 13. Two of these frames had been in hive No. 2 at the Flagg orchards in 1932. The second nucleus hive was made up of frames containing honey, brood, and bees taken from another vigorous noninfested colony.

Hive No. 2 was infested at 6 p. m., April 19, by pouring into it 500

cubic centimeters of a mixture of the two isolations of the blight organism growing in bouillon. The hive was completely covered with this material, the combs, frames and bees being soaked, and the excess liquid was permitted to drain out of the hive. A portion of this bouillon culture injected into young growing apple twigs produced typical blight symptoms.

On April 21, these 2 infested hives were placed beneath 2 Yellow Transparent apple trees that had been enclosed by bee-tight cheesecloth cages, and the bees released. Hive No. 1 was placed beneath tree No. 1. Hive No. 2 was placed beneath tree No. 2. The bees were confined in these cages for 7 days. The trees were in full bloom on April 24 and the results were checked on May 12. On May 2 these hives were taken to the Adams Fruit Farm near Columbus and placed beneath 2 enclosed Grimes Golden apple trees, hive No. 1 being placed beneath tree No. 3 and hive No. 2 beneath tree No. 4. The bees were confined within these cages until May 8, when they were returned to the University apiary for further tests. These trees were in full bloom on May 4 and the results obtained on May 22 are presented in table 2.

It will be observed that no blight occurred in any of the caged trees used in this experiment, although the trees were enclosed in cheesecloth cages containing a beehive that had been infested with the fire-blight organism. The absence of blight in the apple trees enclosed in cages with infested hives in the tests conducted in 1932 and 1933 indicates that bees did not carry the blight organism from the hive to the apple blossoms.

The possibility of the beehive being a source of primary fire-blight inoculum in the spring is directly dependent upon the longevity of the blight organism in the hive (on the honeycomb, frames, or in the honey). That the organism may be able in some way to live over winter in or on bodies of the bees themselves appears improbable, because the bees that are active during the spring period are reared the previous autumn and would have no opportunity to come in contact with the fire-blight organism unless it were present in the hive.

It is evident from the results of the 1932 season's cage tests that the honeybee is able to disseminate the fire-blight organism from one blighted blossom cluster to another and probably from the blighted blossom clusters into the hive in the loads of nectar carried by the worker bees. Gossard and Walton (1) made a series of inoculations both before and after bloom with the mouth parts of bees collected at the entrance of the hive. These mouth parts were inserted into cuts made in young apple shoots. They found that several apparent cases of fire blight were produced in this way. They were unable, however, to recover the blight organism from the dead and dried twigs. Their results indicate that the blight organism is carried

TABLE 2.—Results of 1933 cage experiments on determination of relation of the honeybee to overwintering and spread of *Bacillus amylovorus*

Tree No.	Location	Method of inoculation	Date bees placed in cage	Date of full bloom	Date bees removed	Date observations made	Healthy blossom clusters	Blighted blossom clusters	Blighted clusters per cent
1	Flagg orchards	Hive No. 1 placed in cage <sup>(1)</sup>	April 21	April 24	April 28	May 12	850	0	0.0
2	Flagg orchards	Hive No. 2 placed in cage <sup>(2)</sup>	April 21	April 24	April 28	May 12	1200	0	0.0
3	Adams Fruit Farm	Hive No. 1 placed in cage <sup>(1)</sup>	May 2	May 4	May 8	May 22	680	0	0.0
4	Adams Fruit Farm	Hive No. 2 placed in cage <sup>(2)</sup>	May 2	May 4	May 8	May 22	450	0	0.0

(1) Hive No. 1 infested April 12 and 13 with a virulent bouillon culture of *B. amylovorus*.  
(2) Hive No. 2 infested April 19 with a virulent bouillon culture of *B. amylovorus*.



into the hive from infected blossoms by the worker bees, and might be expected to be present in the hive during the blossoming period.

In an attempt to obtain additional information on this phase of the problem, dilution plates were prepared in September, 1932, from the honey, fragments of comb, and bits of wax taken from the frames used in the hives at the Flagg orchards. All attempts to demonstrate the presence of the fire-blight bacteria in this material resulted in failure. In April, 1933, an attempt was made to isolate the fire-blight organism from honey and fragments of wax taken from the hives used at the Mantle and Mantle fruit farm in 1932. As in the preceding case, we were unable to demonstrate the presence of *Bacillus amylovorus* in this material.

Samples of honey and comb and fragments of wax from the frame bars were taken from hives 1 and 2 on May 8, 1933, after they were returned to the university apiary. Four samples were taken from each hive with sterile instruments and placed directly into tubes of sterile bouillon. This material was allowed to incubate for 24 to 36 hours, and then inoculations were made by injecting portions of the culture into young growing apple shoots. Ten to 25 inoculations were made from each incubated sample. Checks were prepared by injecting sterile water into a similar number of apple shoots.

A further check on the presence of small numbers of the fire-blight organism was made by preparing agar dilution plates from a portion of the material used to inoculate the young apple shoots. After a sufficient growth period, transfers were made from colonies that resembled *Bacillus amylovorus* to tubes of sterile bouillon, incubated for a period of 24 to 36 hours and inoculated into young apple shoots. The presence of the fire-blight organism in the samples taken from these hives could not be demonstrated. All succeeding attempts to establish the presence of *B. amylovorus* followed the procedure just outlined.

On May 9, hive No. 2 was reinfested thoroughly with a heavy suspension of *Bacillus amylovorus* in sterile water. This suspension was applied to the entire contents of the hive with a hypodermic syringe, and directed into the empty cells of the comb, into the cells partly filled with nectar and honey, over the honey cells, and over the bees and frames. About 400 cubic centimeters of the suspension were used on the 3 frames of this hive. A portion of this inoculum produced characteristic symptoms of fire-blight when injected into young growing shoots of apple trees. Checks prepared at the same time remained healthy.

Beginning May 10, samples of honey from the open and closed cells, pieces of the comb, and bits of wax from the frame bars were collected from this hive and from hive No. 1 that was not reinfested. Four samples were collected from each hive daily until May 16, when the experiment

was terminated. These cultures were tested as previously outlined, but none of the inoculated apple shoots developed symptoms of blight. These results indicate that the blight organism probably was unable to remain viable for even 24 hours in this beehive. The results of these tests differ greatly from the findings of other workers, Gossard and Walton (1) and Thomas (8), who found the blight organism viable much longer in the honey cells and on the comb. It should be remembered, however, that their work was carried on in the laboratory, while the infested combs used in this experiment remained in the beehive.

Rosen (6) made numerous isolations from beehives located in an infested orchard. In 1929-30 isolation of *Bacillus amylovorus* from honey plus comb and from bees was reported as successful in 20 injections of young apple shoots out of a total of 2157. In 1931-33 inclusive 1277 inoculations were made with similar material and only one infection is reported. Even if the fire-blight pathogen be present in an occasional beehive, it does not follow that bees will carry this inoculum into apple blossoms and thus cause blight epiphytotics. In the cage experiments reported in tables 1 and 2 of this paper 6957 blossom clusters were exposed to inoculation by bees from hives heavily infested with *B. amylovorus*. Only 9 clusters blighted and there were other sources of inoculum for these blossoms. Even if 9 blossom clusters out of 6957 were infected from beehive material, no orchardist would consider this a serious source of inoculum.

In an attempt to determine whether the blight organism was present on the insects, bees were collected from both hives on May 8. Beginning May 9, when hive No. 2 was reinfested, bee samples were taken from this hive only.

An average of 25 bees was collected daily from the combs with sterile forceps and killed by crushing the thorax. The heads were removed and placed in tubes of sterile bouillon, 5 or 6 heads per tube. The bodies were placed in other tubes of bouillon, 3 or 4 bodies being placed in each tube. Tests for the presence of *Bacillus amylovorus* were made in the manner previously described.

The inoculated shoots were inspected 7 to 10 days after inoculation and the percentage of blight recorded. Apple shoots inoculated with bouillon, in which the heads of bees taken from both hives on May 8 were incubated, remained healthy. Two apple shoots out of 50, which were inoculated with bouillon in which heads taken from hive No. 2 on May 10 and 11 were incubated, developed blight symptoms. Inoculum prepared from this hive after May 11 gave negative results. Bouillon in which bee bodies were incubated failed to produce blight symptoms at all times.

The results of these tests indicate that the blight organism is able to

remain viable for at least 2 days on the heads of bees in an infested beehive, but it was not obtained from the honey or comb 24 hours after it was placed in the hive.

#### LONGEVITY OF *BACILLUS AMYLOVORUS* IN PURE HONEY

Gossard and Walton (1) found in 1915 that the bacteria remained viable for 47 hours in honey. In 1916 they repeated the work and found that the organism was able to survive for 72 hours in honey and perhaps longer, since the experiment was discontinued after that length of time.

Thomas (8) reports that virulent fire-blight bacteria were isolated from an infested comb at every attempt up to 55 days, when the experiment was terminated. He also was able to isolate viable fire-blight bacteria from honey in the comb 15 days after it was infested with the organism.

Pure honey in the comb was artificially infested, in the laboratory, with the fire-blight organism as follows: (a) Transfers of *Bacillus amylovorus* were made from colonies of the organism growing on agar slants to the honey in the cells by means of a needle. The bacteria were mixed with the honey as completely as possible. Several transfers were made to each cell. (b) Other cells in the same comb were infested by injecting a bacterial suspension into the honey in these cells by means of a hypodermic needle. The needle was thrust through the cell sap and into the honey. As the needle was withdrawn, the suspension was expelled and mixed throughout the contents of the cell. Small droplets of this suspension, which oozed out of the cell through the opening made in the cap by the needle, streamed down over the cells below, thus infesting the outside of the comb.

Samples, consisting of loops of honey and bits of the cell walls and caps, were taken at 24-hour intervals for 16 days and tested for the presence of *Bacillus amylovorus*.

Apple shoots inoculated with material as described under (a), for 11 consecutive days, developed the characteristic symptoms of fire-blight. Thereafter all results were negative. Inoculations made from honey infested with the bacterial suspension as described under (b) indicate that the organism remained viable in this material for 9 days but not longer. The results of this experiment indicate that *Bacillus amylovorus* was able to survive much longer in honey or on combs in the laboratory than in the beehive.

On May 9, 3 to 4 cubic centimeters of pure honey were placed in each of 4 sterile test tubes. This honey was infested with fire-blight bacteria that were mixed thoroughly with the honey by means of the transfer needle. Two different isolations of the organism were used in this experiment, two tubes of honey being infested with each one. Transfers were made from the infested honey to bouillon each day for 7 days. All samples of this

honey taken for 5 consecutive days after infestation produced blight in each branch inoculated with it—from 10 to 20 for each test. Thereafter tests failed to give infection.

As a further check on this problem a solution of dextrose and levulose was prepared that duplicated the sugar content of apple honey as given in the "ABC and XYZ of Bee Culture" (4). This solution consisted of 31.6 per cent dextrose and 42.00 per cent levulose. Tubes containing ten cubic centimeters of this sugar solution were infested with *Bacillus amylovorus* taken from agar-slant colonies. Samples of this material were transferred to tubes of sterile bouillon daily for 12 days and handled in the manner previously described for the tests with honey.

Samples of this solution collected the first 5 days after it was infested with the blight organism produced blight when injected into young growing apple shoots. The results of all later tests were negative. The effects of this sugar solution on the blight organism were apparently similar to the effects of pure honey, since viable organisms were not obtained from either the honey or the sugar solution after 5 days.

Of interest in this connection are numerous experiments conducted by Sackett (7). Some of his results are as follows: *Bacillus fecalis alkali-genes*, dead in 5 hours; *B. dysenteriae*, dead after 10 hours; *B. paratyphosus*, dead after 24 hours; *B. enteritidis* and *B. typhosus*, dead in 48 hours; *B. lactis aerogenes*, *B. proteus vulgaris* and *B. suispestifer* dead in 4 days, and *B. coli communis* dead on the 5th day.

Bacteriologists generally consider these organisms to be nonspore forming. Since the fire-blight bacillus is not known to form spores it may be considered to be in the vegetative state when it enters the hive in a load of nectar and when the nectar is converted into honey by the bees.

The results of other workers as given previously indicate that the life of *Bacillus amylovorus* in pure honey does not greatly exceed the longevity of other similar vegetative organisms in the same medium. This fact further indicates that the honey cells are not a probable source of fire-blight inoculum in the spring.

#### SUMMARY

Honeybees carried fire-blight bacteria from artificially inoculated blossom clusters on the lowermost branches to other blossom clusters on the same tree.

Beehives that had been infested with virulent cultures of *Bacillus amylovorus* were placed under apple trees enclosed within cheesecloth cages. Bees did not carry blight inoculum from the hive to the blossom clusters.

The transfer of bee colonies from one locality to another did not spread

the fire-blight organism, even though these colonies had been confined to trees badly infected with blossom blight.

The longevity of *B. amylovorus* in pure honey was found to vary from 5 to 11 days. The organism was recovered for 5 days from inoculated honey in test tubes, for 9 days from honey in the comb inoculated with a suspension of the organism, and for 11 days from honey in the comb that had been inoculated with fire-blight bacteria transferred from agar-slant colonies.

It was impossible to demonstrate the presence of *B. amylovorus* on the combs, frames, or in the honey in a beehive 24 hours after it was infested with a suspension of the organism in a virulent state.

*B. amylovorus* was obtained from the heads of honeybees taken from a hive 2 days after it was infested with a suspension of the virulent organism. Thereafter it could not be demonstrated that the blight organism was present on the insects.

The results of this investigation indicate that the beehive is not a probable source of fire-blight inoculum in the spring, under Ohio conditions.

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# PERONOSPORA VICIAE AND INTERNAL PROLIFERATION IN PEA PODS<sup>1</sup>

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## INTRODUCTION

During the growing seasons of 1932-33 certain growers of market peas in California were concerned with the occurrence of chlorotic lesions and velvety internal proliferations in green pods of pea, *Pisum sativum* L. Externally, a yellowish blotch generally denoted the condition, while internally, a mealy coating of mycelium and oospores lined the cavity, or there was a felty proliferation of the inner pod membrane accompanied by oospores (Fig. 1). Sometimes the felty condition seemed to be unattended by superficial fungus growth, although still associated with the yellow blotches, and the oospores were found in the ovary wall tissues between the surface blemish and the membrane bearing the internal proliferation. Although other causes of a proliferation of the inner epithelial lining of pea pods are known (9), the present discussion is limited to proliferation that accompanies oospores.

Pod infection by downy mildew has been reported in the literature, but the internal proliferation of the pods that has been found associated with oospores in certain coastal districts of pea culture in California apparently has not been described. W. G. Smith (8), in England, noted *Peronospora viciae* (Berk.) de Bary, growing within the pods of garden peas and upon the seeds. Linford (5) referred to the yellowish white, porcelanous, slightly swollen areas on leaves, stems and pods in which oospores of the fungus are developed and noted an occasional systemic infection. Melhus (6) found mycelium in the seed coats of seeds from infected pods. In the spring of 1931 Ramsey (7) noted "what appears to be the oospore stage of *Peronospora*" on pods in a shipment of peas from Santa Maria, California. Heald (4) mentioned pod infection in Washington as a serious aspect of the disease, responsible for 60 per cent of culls in certain pickings, and found oospores in seed from such pods. The symptom of internal proliferation of the pea pod in association with oospores, is reported by Sorauer (10) in his chapter on intumescences. He observed that the inner parchment-like lining sometimes forms intumescences resembling mold in their white felty appearance, and that in one case he found in the intumescence tissue numer-

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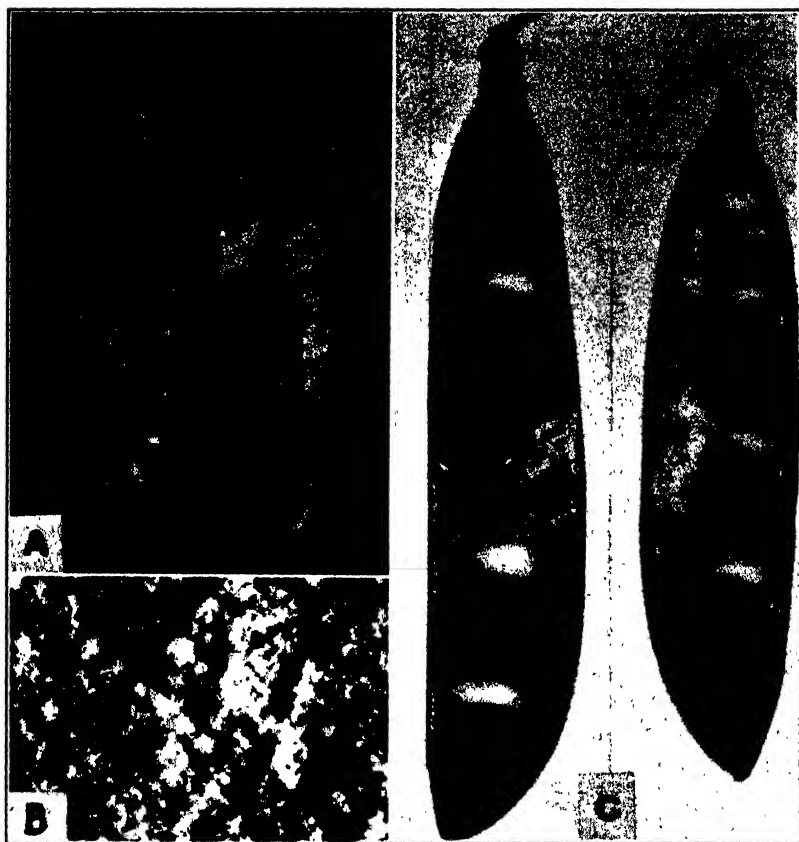


FIG. 1. A. Thin mealy layer of mycelium and oospores of *Peronospora viciae* coating the interior surface of a pea pod artificially inoculated by atomizing a plant bearing young pods, with a conidial suspension. Also typical of some natural infections in the field. B. Mycelium- and oospore-covered pod lining shown in A.  $\times 22$ . C. White, felty, mold-like patches of epithelial proliferation sometimes found associated with infections of the pod wall by *Peronospora viciae*.

ous oospores that "presumably belong to *Peronospora viciae*." Sorauer attributed the cause of the intumescences, however, to environmental influences.

#### SYMPTOMS

The external symptoms, already briefly referred to, usually constitute the least conspicuous phase of the disease, yet it is upon the detection of these surface blemishes that packers depend in culling out pods unsuited for market. The yellowish blotches,  $\frac{1}{4}$  to 1 inch or more, in extent, tend to become slightly sunken and may be homogeneous, or heterogeneous with the blotch, consisting of many small yellowish islands.

A common type of internal symptom is the presence of white, felty, mold-like patches of epithelial proliferation, occurring directly under the external yellowish blotches (Fig. 1, C). This felty layer is composed of living epithelial cells in which chloroplasts and other cell structures are visible. The delicate mat-like growth frequently involves the greater part of the membranous lining, extending  $\frac{1}{8}$  inch or more into the pod cavity, and envelops the ovules. The hair-like outgrowths are several cells long, curl apically and intertwine, producing in mass the dense felty layer. Although no fungus may be evident upon either the exterior or interior surfaces of pods showing these symptoms, yet, peeling back the internal parchment-like lining, masses of oospores are found imbedded in the ovary wall directly below the velvety growth.

Where the fungus is not confined within the wall tissues, but has pushed through the inner membrane into the pod cavity, and where no proliferation occurs, the thick, nonseptate mycelium of varying diameter produces enormous numbers of oospores as it spreads over the interior surface of the pod. This thin, white coating of mycelium and oospores, which may give a mealy appearance to the entire lining of the pod cavity, resembles a dusting with flour (Fig. 1, A, B). As the oospores mature, the color of the layer changes to a cream-yellow, then brown. When such invasion takes place early in the development of the pod, the ovules generally fail to mature, and the pods are empty. Frequently, however, the ovules mature and microscopic examination has shown that the fungus may enter the seed coats.

In a combination of the proliferation of the epithelial cells and the development of the fungus in the pod cavity, the felty mat gradually assumes a brownish discoloration with the development of mature oospores scattered through it, and apparently also as a result of death of the proliferated cells evidently caused by the fungus, which may be observed twined about the hair-like growths. Here again the ovules frequently mature without any apparent abnormality.

#### INOCULATION TRIALS

Since there was uncertainty as to the identity of the fungus producing the oospores, because fields yielding quantities of oospore-infected pods frequently showed almost no downy mildew upon the foliage, attempts were made to obtain the conidial stage of the fungus. Affected pods were placed in moist chambers held at 15° C. and at room temperature, but no fungus outgrowth resulted. Limited attempts under similar conditions to germinate oospores upon healthy, freshly picked pods, and to infect living pods on potted plants in the greenhouse by introducing oospores and mycelium into the ovary cavity were also unsuccessful.



In late October, 1932, a number of pea pods heavily infected with oospores were chopped up and incorporated in the upper part of the soil in half a dozen 6-inch pots. Pea seed of the variety Hundredfold were planted and the pots, along with controls, placed outdoors. No sign of disease developed upon the resulting plants, which were killed by frost in December. In mid-January, 1933, the same pots were replanted in the greenhouse with seed of the variety Laxton's Progress. When the plants came into bloom near the first of March, no disease was apparent, and two of the pots were placed in the saturated atmosphere of a large glass humidity chamber, with several control pots. On examination 6 days later the conidial stage of a *Peronospora* was found on the stems in a zone extending 2 inches above the soil. None of the plants in the control pots showed any sporulation. One of the pots of infested soil contained 9 plants, 7 of which bore stem infection of downy mildew, and in the other pot, 5 of 6 plants were similarly diseased. A week later the conidial fructification extended to a height of 4 to 5 inches above the ground line, and one small plant, apparently from a seed that had germinated more slowly than the rest, had died, as a result of a systemic infection. In contrast with the sparse sporulation on the plants with infected stems, the systemically infected seedling was densely covered with the velvety, lavender gray mildew. This indicates, although it does not prove, that systemic infection of peas with downy mildew may occur where seed is planted in oospore-infested soil. No other infection developed either in the greenhouse or outdoors, although peas had been grown in the greenhouse during this period continuously. There was no reason to suspect that the infection could have come from any source other than the diseased pods placed in the soil.

Conidia obtained from the stem sporulation on the plants that had become infected from oospores in the soil, were used to inoculate other plants of the same variety. Four pots averaging 7 pea plants each, all in the young-pod stage, were atomized with a suspension of conidia, kept in a humidity chamber 2 days, and then placed outdoors with several pots of noninoculated plants as controls. Within 2 weeks definite symptoms were apparent upon the inoculated plants in the nature of a slight yellowing and dwarfing of the foliage and yellowish blotches on the pods, the latter typical of the blotches on the oospore-infected pods such as had been used in the original soil inoculation. Two of the 4 pots of peas showing symptoms of infection, but devoid of any external growth of the fungus, were stored for about 18 hours in the saturated atmosphere of a humidity chamber, along with 2 pots of the control plants. At the end of this time the young foliage, stems and petioles of several inoculated plants were covered with the characteristic lavender gray masses of conidiophores and spores of *Peronospora*, whereas no evidence of infection was found on the controls.

Furthermore, a few of the young green pods showed yellowish blotches and bore conidiophores and conidia, although the sporulation was more sparse than upon the foliage. The interior of these pods was found to be coated with oospores and mycelium, as previously described for field specimens (Fig. 1, A). However, epithelial proliferation had not occurred to any noticeable extent, a situation also frequently found in field infections when the fungus entered early in the development of the pod.

The 2 pots of peas that showed symptoms of infection, but had not been placed in the humidity chamber with those just described, had meanwhile been subjected to several cloudy, somewhat humid days outdoors, and upon the smallest, youngest, yellowed leaflets a light, whitish coating had appeared upon the surface. Microscopic examination of the whitish coating proved it to be similar to the mycelial and oosporic coating that had been found on the interior of the infected pods. Apparently the humidity, or some other factor, was sufficiently favorable to encourage the development of mycelium and oospores upon the leaf surface, but not enough to bring about conidial sporulation. Upon placing these 2 pots in the moist chamber along with adequate controls, abundant conidial sporulation occurred.

Beginning, then, with oospores from infected pods it was possible to infect pea plants through the inoculation of soil and to recover the conidial stage with which in turn both the conidial and oospore stages were produced upon the leaves, stems, and pods of still other plants.

Microscopic examination showed that the fungus agreed within reasonable limits with de Bary's (1, p. 112-113) description of *Peronospora viciae*, and also with the measurements given by Gäumann (3) for both *P. pisi* and *P. viciae*. However, since Gäumann reports the oospores of *P. pisi* unknown, and the oospores here described in pea pods seem to compare favorably with those of *P. viciae*, it seems desirable at present to refer to this fungus by the latter name.

#### FIELD OBSERVATIONS

Market peas grown in Alameda, Monterey, and San Luis Obispo Counties, California, are subjected to a cool humid climate. Even though the days may be warm, the nights are cool, and the plantings are exposed to frequent ocean fogs. Peas are grown extensively in these regions and the growing seasons are long, a condition favorable to the establishment and perpetuation of disease-producing organisms. In these regions downy mildew is common on the foliage during the spring, and often severe. Systemically infected plants occurring at this season suggest either seed infection or seedling infection from infested soil, the conidial masses usually breaking out over the entire plant before the blossoming period. From such infection centers secondary infections of the foliage spread through

the remainder of the field. Abundant inoculum is undoubtedly provided to the soil by infected crops in the form of oospores in plant debris. The fall crop of peas, which may be planted after July, is grown under irrigation and is subject to rains only late in the fall, whereas, with the spring crop, rains occur early and decrease with the approach of blossoming. In the 1932-33 seasons foliage infection during the early fall crop was much less abundant than in the spring crop, yet pod infection was much more troublesome in the early fall crop.

Although oospore-infected pods have been found both in the spring crops and fall crops, there appeared to be a greater amount of pod infection in the fall and the conspicuous symptom of epithelial proliferation was prominent only during the late summer and early fall. Why proliferation should have been so much more abundant on pod lesions in the early fall crop than in the spring crop is perhaps tied up with the environmental complex of temperature, humidity and light, as suggested by Curzi and Barbaini (2) for intumescences occurring on the exterior surfaces of pea pods. It is during this period that plants are subjected to alternate periods of high summer temperatures and cool drenching fogs. It seems that under these conditions of growth the oosporic lesion provided the stimulus for the proliferation of the cells of the membrane on the interior of the pod.

In 1933 the felty pod condition was first observed in a field in Monterey County on peas being picked in mid-August. Casual observation indicated that the field was free from downy mildew, but careful search revealed very small, scattered lesions on the leaves and stipules, bearing only sparse and occasional sporulation. The diseased pods showed the typical yellowish, rather inconspicuous surface blotches. Examination of a number of these showed that within each pod there was a felty layer, ranging up to an inch in extent, and occurring directly under the external blotch. No fungous growth was observed either on the outer surface or in connection with the proliferation, but, on peeling back the felt-bearing membrane, a mass of oospores was found imbedded in the tissue of the pod wall. These lesions were relatively recent, as indicated by their size, and their occurrence was preceded by an extended period of very foggy weather.

It appears from the experiments and observations discussed, that the oospores found in pea pods showing the symptoms described, are those of downy mildew, *Peronospora viciae*. Also it seems that under certain conditions, the presence of the mycelium and oospores of *P. viciae* in the ovary wall may be accompanied by internal proliferations of the inner epithelial membrane, the conspicuous symptoms of which are here described. Although the identity of the oospores has been established experimentally, the suggested relation of epithelial proliferation to the presence of *P. viciae* in the ovary wall remains based upon circumstantial evidence. Repeated

observations of the character already referred to emphasize the probability of the relationship, but experimental methods, taking into account the peculiar environmental factors, will be necessary to prove that *P. viciae* is the cause of this type of cell stimulation. It should be recalled, also, that felty pod is known to occur in response to inciting agencies other than this fungus, both *Cladosporium pisicola* Snyder, and wounds already having been demonstrated as capable of bringing about the phenomenon.

#### SUMMARY

During certain periods, pods of market peas, *Pisum sativum*, grown in coastal areas of California, sometimes show high percentages of oosporic lesions, unattended by appreciable amounts of foliage infection of downy mildew.

Externally, the infection produces inconspicuous, yellowish blotches. The fungus may remain confined within the pod-wall tissues, in which case it is frequently attended, internally, by a felty proliferation of the epithelial lining, beneath which the oospores lie imbedded, or, the fungus may enter the pod cavity and spread over the inner surface as a mealy layer of mycelium and oospores, in which case the internal intumescences may or may not accompany the fungus.

Incubation of the diseased pods under various conditions has failed to bring about conidial sporulation, and likewise, attempts to infect living pods by direct inoculation with oospores and mycelium have failed.

The conidial stage of *Peronospora viciae* has been obtained, however, by growing plants in soil artificially infested with oospores from diseased pods. With conidia thus produced, abundant infection of healthy plants was obtained yielding both the common downy mildew sporulation on the foliage, the yellowish blotches on the pods, and oospores in the tissues of both diseased foliage and pods.

Incubation of these artificially infected pods in a humidity chamber has yielded a sparse development of conidial sporulation upon the pod surface. Internally such pods have shown a thin white or tan coating of mycelium and oospores lining the pod cavity.

An instance of systemic infection in a seedling grown in oospore-infested soil suggests that a part at least of the systemic infections observed in the field may arise as a result of soil infestation. Also, the presence of mycelium in the seed coats of seed from oospore-infected pods makes possible dissemination of the fungus with seed.

Under certain conditions a superficial layer of mycelium and oospores was produced as a white coating on the youngest leaves, instead of the conidia ordinarily observed under more humid conditions, and resembled the peculiar adaptation to surface growth of the mycelium and oospores

frequently found in the pod cavity spreading superficially over the inner membrane.

Field observations have shown a close association, during certain periods, between white, mold-like, internal intumescences extending into the pod cavity and oospore-containing lesions of the ovary wall, the only external symptom being a yellowish blotch on the pod surface. It is suggested that under certain conditions the infection in the pod wall provides the stimulus for the inward proliferation of cells constituting the internal epithelial membrane. Other agencies are known to incite a similar proliferation.

The observation that pod infection and associated internal intumescences appear most abundantly following heavy coastal fogs presupposes that this environmental factor provides the opportunity for infection and also favors the development of the intumescences when the necessary stimulus is present.

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# THE CONFINEMENT OF CHLOROPICRIN AND OTHER GASES FOR FUMIGATION PURPOSES<sup>1</sup>

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In connection with studies on soil fumigation for nematode control reported elsewhere (2, 3, 4), it was found that chloropicrin was efficient in proportion to the efficiency with which it was confined in the soil. Experiments in which the opportunity was available for it to evaporate quickly gave very poor results in nematode control, whereas cases in which the gas was confined by means of a fairly gas-tight cover gave excellent control. These observations led to studies on the penetrability of the gas through covering materials of various kinds. Details and results of these studies are herein reported.

## METHODS

The method used for the study of permeability of membranes to chloropicrin gas was a purely empirical one, consisting of the insertion of the chemical in liquid form into ordinary 4-inch Petri dishes covered with the membrane sealed tightly in place at the edges, and then determining the rate of evaporation by reduction in weight. Any kind of paper, even cheap newsprint paper, proved satisfactory as a base upon which to apply coatings of the different materials to create the experimental membranes. Eventually, however, 40-pound Kraft paper was used almost exclusively because of its strength and freedom from troublesome curling.

Detailed procedure was as follows: The paper for the cover, cut in circles of about  $\frac{1}{2}$  inch greater diameter than the dishes, was glued tightly in place, well over the edges. The cover was then painted with the coating material in liquid form and the dishes were set aside for the coatings to dry. The attainment of constant weight was considered as sufficient drying. Variations of a few milligrams thereafter, with changes in air humidity, were not sufficient to alter major conclusions. Chloropicrin was inserted by means of a burette with capillary outlet through a pinhole made in the cover. As a rule, exactly 5 grams were so inserted. Slight adjustments to bring about exactness were made with the dish on the balance by means of a small capillary medicine dropper. Immediately thereafter the pinhole was covered with a small piece of adhesive paper that was then coated with the same material used on the cover. The dishes were laid aside and weighed at frequent intervals and losses in weight due to evap-

<sup>1</sup> Published with the approval of the Director as Technical Paper No. 59 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

oration of the chemical were recorded in decigrams. With the initial weight of the chemical exactly 5 grams these figures were converted readily to percentage loss by multiplying by 2.

The temperatures of exposure throughout the tests were fairly uniform, ranging between 22° and 28° C. with an average of about 25°. It is recognized that the vapor pressure of chloropicrin, as of other volatile compounds, varies greatly with the temperature and that more reliable results would have been attained had a constant temperature been maintained. Lacking equipment for this, the writer had to be content with his records that showed satisfactorily the wide differences in permeability of different coatings, and permitted him to select those of highest efficiency for confining the gas. At 25° C. the vapor pressure of chloropicrin is 23.97 mm. (1). This pressure, except for the greater or smaller variations due to temperature changes (maximum range, 20 to 28 mm.), may be considered to have remained relatively constant in the small chamber used (about 80 cubic centimeters capacity) until the liquid inserted had completely evaporated. Any partly pervious membrane used as a cover should theoretically permit a fairly uniform rate of penetration of the gas. With a very permeable membrane the rate of loss may be expected to be rapid; with a highly impervious one the rate is slow. Paper covers alone permitted of very rapid evaporation, the material disappearing completely as a rule in about 2 hours.

#### MATERIALS USED AND RESULTS

As with the method, so with the choice of materials, procedure was at first purely empirical. Various materials were tested as paper coatings, with widely varying results. Paints and varnishes of various kinds were found to be relatively inefficient. Losses of 90 per cent or more occurred in less than 24 hours with coatings of oil house paints, Valspar varnish, amyl lacquer, a commercial black enamel, a graphite roof paint, "Convolve" rubber, and various kinds of rubber latex. Ordinary building cement and a waterproof building cement showed total loss in 2 to 4 hours. Shellac showed 88 per cent loss in 24 hours. It was arbitrarily considered that if 50 per cent loss occurred in 48 hours or less the material used might well be eliminated from further consideration. This, of course, threw out all those just mentioned.

Cellophane, used in place of paper, was found to be highly efficient for confining chloropicrin. Aside from its uncertain durability for the purpose proposed this material is far too expensive for practical use. Its efficiency, however, led to tests with cellulose acetate preparations. Coatings of Celanese on paper in a very thin layer showed only 26 per cent loss (13 decigrams of the original 50) in 48 hours, 40 per cent in 72 hours, and

64 per cent in 6 days. A slightly heavier application showed somewhat greater efficiency. While fairly effective for the purpose in mind, this material, also, is too expensive for general use.

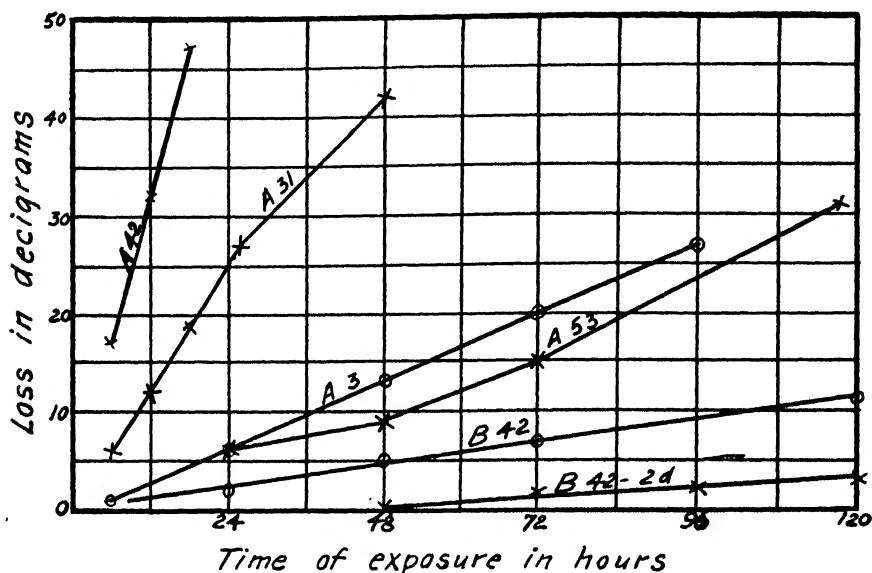


FIG. 1. Graph showing loss of chloropicrin from 4-inch Petri dishes covered and sealed with several different membranes. A42, a representative tar-impregnated mulching paper. A31, paper coated with shellac. A3, Celanese, very thin application; note uniform rate of loss. A53, water glass; note more rapid loss after 48 hours, due to development of very fine crackings. When dishes were placed out of doors, subject to greater fluctuations of temperature and moisture, cracks developed quickly and total loss occurred in a few hours. B42, Golden Ground hoof and horn glue. B42—2d, same dish repeated 6 weeks later, showing apparent improvement with age. The carbon bisulphide line with membrane B126, Cascin glue, would fall just above that for B42, with 18 decigrams loss in 120 hours. All other covers tested showed losses of over 50 per cent in less than 64 hours (all but one in less than 36).

Sodium silicate (water glass) proved to be very highly effective as a paper sizing material in laboratory tests, as described. Its efficiency, in comparison with other materials, is graphically shown in figure 1. Its cost, moreover, is very low. Water glass, however, has the serious disadvantage of cracking badly when exposed to temperatures and humidities of high variability. This cracking leads to immediate inefficiency for holding chloropicrin. Unless this can be overcome, water glass-coated papers are completely impracticable for confining fumigants more than a few hours.

Of all the materials tested, certain adhesive hydrophyllic colloidal substances, including glues of various kinds, starch paste, and gelatine, proved very highly efficient for holding chloropicrin. Actual results obtained



with some of the better applications are shown in table 1. Some of the results with less efficient materials also are given by way of comparison. Data on tests of the penetrability of the usual pineapple-field, tar-impregnated mulching papers likewise are shown.

It is well to note, in interpreting this table, that the original quantity

TABLE 1.—Rate of penetration of chloropicrin gas through various covers. Standard conditions, 5 grams chloropicrin in 4-inch Petri dish, covers glued tightly in place; dishes held at room temperature, averaging 25° C.

Test No.	Covering material	Loss of Chloropicrin, in decigrams							
		Hours							
		6	12	18	24	48	72	96	120
A38	40-lb. Kraft paper . . . . .	Completely evaporated in less than 2 hours							
A39	Pabco #104 Mulch paper . .	10	23	36	50				
A40	“ #107 “ “ . . . . .	8	16	24	28	46			
A42	“ #T140 “ “ . . . . .	17	32	47	50				
A29	Greenhouse paint . . . . .	17	35	50					
A31	Shellac, thin . . . . .	6	12	19	26	42			
A33	“Convole” rubber . . . . .	50							
A36	Valspar varnish . . . . .	50							
A19	Paraffin . . . . .	9			16	26			
A3	Celanese . . . . .	1			6	13	20	27	
A52	Water glass . . . . .	2			6	13	22		39
A53	“ . . . . .	1			6	9	15		31
A55	“ . . . . .	1.5			8	12	16		26
B6	Water glass . . . . .	1			4		11		
B7	“ (exposed to sun) . . . . .		47						
B8	“ “ “ “ . . . . .		47.5						
B42	Golden Ground glue . . . . .	0			2	5	7		11
“	Same dish, 6 wks. later . . . . .	0			0	0	2	2	3
B44	Golden Ground glue . . . . .	1			3.5	9.5	11	14	17
“	Same dish, 6 wks. later . . . . .				0	1	4	5	9
B53	Glue + Ebonol . . . . .				0	2.5	4.5	5	
B56	“ “ . . . . .				1	3	6	15	
B67	Glue + water glass, 10 per cent . . . . .				4	6	16		16
B79	Casein glue . . . . .				0	0	2	3	4
B80	“ . . . . .				0	0	3	6	9
B105	Peerless Ground glue + formaldehyde . . . . .				0	0	3	7	8
B142	U. S. Govt. Waterproof glue . . . . .				3	12	13	15	21
B147	“ “ . . . . .				0	6	9	14	18
B151	Starch paste . . . . .				1	7	10		20
B154	Gelatine . . . . .				0	4	11	15	18

of chloropicrin inserted was always 5 grams. The readings of losses, in decigrams, therefore, give an immediate picture of the proportion of the chemical that has disappeared at any particular time. Multiplying the figures by 2 gives this proportion in terms of percentages. Scanning the 48-hour column, for example, shows the covers that were poor and those that were efficient in holding the chemical up to that period. The figures in the final column show the very high efficiency of some of the materials used, with less than 10 per cent loss indicated in 5 days of exposure.

The table shows only a few representatives of each of the various lots of material tested. The mulching papers used in pineapple-plantation practice, while certainly holding the gas to some extent, were not highly efficient for the purpose, with indicated losses of 100 per cent or nearly so in 48 hours or less. The second group in the table shows the very low order of efficiency of paints, varnishes, rubber coatings, and paraffin. Various other materials of similar natures were tested with like results. The third group includes materials of apparently high efficiency but objectionable from other points of view, such as cost or durability. The final group, B42 to B154, includes the range of materials tested that show, as a rule, high efficiency for retaining chloropicrin, and that are otherwise practicable.

In an effort to develop a coating that would make the paper more or less weatherproof as well as gas-impervious, combinations of various materials were tested. Some of the U. S. Forest Products Laboratory waterproof glues (5) proved to be highly retentive of the gas. Ordinary glues sprayed or brushed with formaldehyde solution were rendered waterproof to some extent, without material change in their permeability. Supplementary coatings of waterproofing materials, such as the tar paint, "Ebonol," did not alter materially the chloropicrin-holding capacity. In test No. B53, in which a light grade of Kraft paper (Graham Paper Co. 15 lb. Kraft) was first treated with Golden Ground animal-product glue, thoroughly dried, and then coated with Ebonol, the loss was virtually nil in 96 hours. Forest products casein glue, formula No. 11 (5) was fairly efficient and, at the same time, somewhat waterproof. Le Page's casein glue, applied as a thin coating, was one of the best treatments used. There was some tendency, however, for the casein glues to peel away from the paper upon exposure, whereas such tendency was entirely lacking in the ordinary hoof-and-horn glues, which, however, are not of themselves so waterproof.

The ordinary hoof-and-horn glues were from many points of view the most practicable of the materials tested. Many different kinds and grades were approximately the same in chloropicrin-holding capacity. An apparent increase in efficiency with age is evident in the comparison of the two

coatings, numbers B42 and B44, with the same two tested 6 weeks later. The lower grades are highly efficient, easy to apply, and inexpensive.

Figure 1 shows graphically the results obtained with representatives of the different materials tested. In general, in accordance with theoretical considerations, the rate of diffusion of chloropicrin through the various unbroken membranes was fairly uniform, and, consequently, the plotted lines are approximately straight. Slight variations are explainable by variations in moisture absorption of the covering material and, of course, by temperature differences. In certain cases, however, deviations from the straight line are quite marked. For example, the water-glass-covered dishes, as a rule, showed more rapid diffusion with the passage of time, and this is indicated in figure 1 by the greater steepness of slope of the lines after the second or third day of exposure. This is due no doubt to the before-mentioned cracking of the water-glass membranes. Exposure of dishes to the sun for an hour or two hastened the cracking and the consequent escape of the gas.

Several of the coated papers were tested for permeability to certain other gases. It was found that carbon bisulphide penetrated most membranes much more readily than did chloropicrin. Figure 1 shows results from a single test. Water glass, rubber, ordinary glue, in fact, most of the materials tested were very inefficient. The Forest Products Laboratory casein glue, interestingly, was the only material tested that was highly efficient. Several repetitions with this glue were equally good. In view of the rather high vapor pressure of this gas (361 mm. at 25° C.) this finding is encouraging as to possibilities. Tetrachlorethane was very much like chloropicrin in its ability to penetrate membranes. Glue coatings of various kinds were efficient to the extent of permitting only very small losses, ranging from 8 to 18 per cent. in the course of 5 days. Hydrocyanic acid gas diffused rather rapidly through practically all the membranes that were tested. No quantitative tests were made with this gas, judgment as to rate of diffusion being based solely on the rate of coloration of Guayacum-copper sulphate test papers. Obvious differences existed, and, inasmuch as this gas is used so extensively in fumigation, further studies would appear to be justified.

#### APPLICATION

In experiments on soil fumigation for nematode control, reported elsewhere (3), the writer has obtained better than 99 per cent control with chloropicrin in a large number of cases in which the soil was confined in closed containers sealed with special covers of gas-impervious papers. Perfect control was obtained in several cases. In nematode-control experiments in pineapple fields in Hawaii (2), strikingly better results were obtained with the use of mulching paper covers over treated soils than when

no covers were used (observations without quantitative data). What might be obtained with a highly impervious cover in place of the less efficient mulching paper (see present results) remains to be determined. For field applications the chemical is first applied in the soil, immediately after which the unbroken paper is applied over the bed with the edges buried as deeply as practicable (6 or more inches if possible) and the soil well firmed over the edges. Wetting the soil at the edge of the paper, where practicable, is another precaution contributory to better results in that it helps to prevent the outward diffusion of gas away from the place it is needed. A tight paper cover definitely makes a difference of several days in gas retention in the soil. For special applications, such as seed-bed or greenhouse-bed fumigation or treatment of isolated spots in a field, the use of a metal or wood frame sunk into the ground around the plot to be treated, with the paper cover tightly sealed to the frame all the way round, is indicated. In one experiment in which this was done with only moderate efficiency (3), excellent results in nematode reduction and improved growth of tomato plants were obtained.

The same gas-impervious paper has been used to advantage for insect control. The writer constructed of wood a light box frame 2 by 2 by 2 feet in dimensions, and covered it with Kraft paper, coated inside and out with glue. A similarly covered lid was so built as to fit tightly into place with the line of junction flush with the surface of the frame. In a practical test with this miniature "fumigation chamber" a small piece of bamboo furniture infested with a small wood-boring beetle (species undetermined) was placed in the box; an open dish containing about 5 cubic centimeters of chloropicrin was inserted and the cover sealed in place with strips of adhesive manila tape. After sealing no odor of chloropicrin was apparent at any time about the box. The lid was removed after one day and a very strong odor of the gas was still apparent in the box. Complete efficiency in killing the insects was attained. Similar fumigation chambers up to 1000 cubic feet capacity have been constructed by the writer, and have been used to practical advantage for the killing of stored-product insects of various kinds.

The technique of applying the gas-impervious coatings is simple, and the costs of the cheaper materials surprisingly low. The writer has applied glue sizings by either brushing or spraying the glue in 12 or 15 per cent concentration (by weight) in warm water. For large-scale applications he has successfully used a dipping process, unrolling 4-foot Kraft paper directly through a specially constructed vat containing the hot liquid glue (10 per cent concentration is satisfactory for this) and lifting it, to dry, with an overhead pulley and rope arrangement. In this way he has frequently treated strips 30 feet long in only a minute or two. One pound

only of a glue retailing at 15¢ per pound was sufficient to coat adequately, on both sides, 500 square feet of paper.

#### SUMMARY

Concurrent with soil-fumigation tests for root-knot-nematode (*Heterodera marioni*) control reported elsewhere, studies were made in the nematology laboratory of the Experiment Station of the Association of Hawaiian Pineapple Canners, at Honolulu, of means of confining fumigation gases to increase their killing efficiency. Tests were conducted by inserting measured quantities of the volatile chemicals used, in liquid form, into 4-inch Petri dishes previously sealed with covers of paper coated with the various membranes to be tested, and recording losses in weight with the passage of time. Paper alone was wholly inefficient in confining the gases. Papers covered with thin continuous films of various adhesive hydrophyllic colloids, particularly glues of various kinds, gelatine, and starch paste, were highly efficient in confining chloropicrin. Cellulose acetate was likewise good. Water glass was highly efficient in the laboratory at relatively uniform temperatures but cracked quickly when exposed to the sun, thereby immediately losing its efficiency. Ordinary animal glue actually improved with age. United States Forest Products Laboratory casein glue was the only material that was highly efficient in confining carbon bisulphide. Tar impregnated commercial mulching papers were very poor in efficiency. Papers covered with oil-containing paints and varnishes were very poor, most of the gas diffusing through them in much less than 24 hours. Animal glue being cheap, durable, and highly efficient for confining chloropicrin, may be used as a sizing on paper to produce a relatively gas-tight covering material applicable for general fumigation purposes. Practical applications with excellent results have been made experimentally in soil fumigation for nematode control, and in grain fumigation for the control of weevils and moths of various kinds.

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# TRICHODERMA SHEATH SPOT OF RICE

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## INTRODUCTION

The disease referred to herein as the "Trichoderma sheath spot" of rice was first called to the writer's attention during the latter part of the summer of 1929 by J. Mitchell Jenkins, of the Rice Experiment Station, Crowley, La. A few miles north of Crowley a field of Blue Rose rice was inspected in which the disease was so severe that all the lower leaves had been killed as a result of the lesions on the sheaths. Subsequently, the disease has been found in different degrees of severity on various commercial varieties of rice in Louisiana and east of the Brazos River in Texas. In the fall of 1932 it was found for the first time in Arkansas, just west of Roe, on Early Prolific rice growing on "new" land.

## SYMPTOMS

The lesions appear first on the sheaths at about the water line, in mid-July. When the lesions first become visible they are reddish brown, 2 to 3 mm. long by about 1 mm. wide. Within a relatively short time, however, they enlarge considerably and their centers become lighter in color ("cream," according to Ridgway<sup>2</sup>) and the margins remain reddish brown ("light seal brown," according to Ridgway). Various stages in the development of the lesions are shown in figure 1.

The central portion of the spot is irregular in outline, owing to the fact that the tissues over the vascular strands do not bleach out so readily as those between the strands. This is shown in the specimen at the extreme right in figure 1. Frequently, when abundant secondary infections appear, the central portion of the spots do not bleach out so readily as in earlier infections, and the whole spot may then vary from a grayish brown at the center to the darker seal brown of the border. As may be seen in the specimen at the extreme right in figure 1, the darkest portion of the border is immediately adjacent to the bleached-out portion. Old lesions frequently attain a length of 10 cm. and, late in the season, may be found on the sheaths from the water line to the base of the flag leaf. In a few cases the fungus has been observed to produce a definite leaf stripe, extending from the base to the tip of the leaf, apparently a continuation of a sheath infection.

<sup>1</sup> Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Investigations conducted in cooperation with the Louisiana, Texas, and Arkansas agricultural experiment stations.

<sup>2</sup> Ridgway, Robert. Color standards and color nomenclature. 53 col. pls., 1115 named colors. Washington, D. C. 1912.

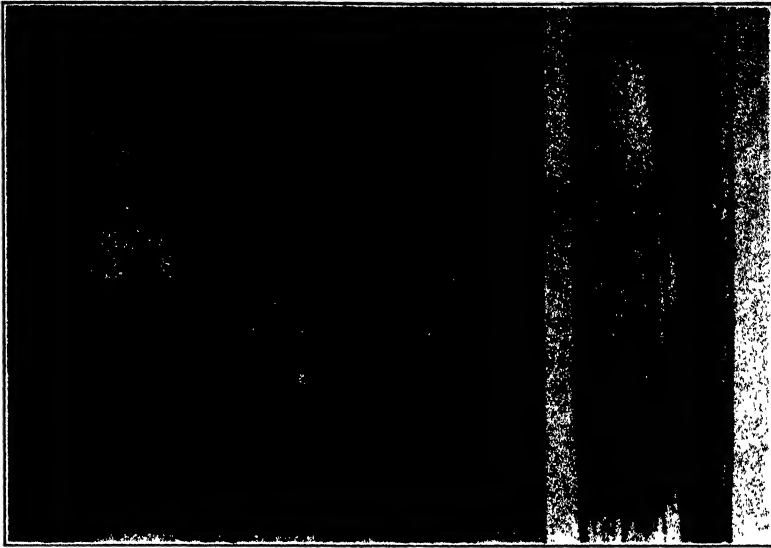


FIG. 1. Various stages in the development of the lesions caused by *Trichoderma lignorum* on rice sheaths. Three specimens at left,  $\times 1$ ; that at right,  $\times 3$ .

#### VARIETAL SUSCEPTIBILITY

A marked difference has been observed in the reaction of varieties to infection by this fungus. The most susceptible varieties, listed in the order of their relative susceptibility, are Vintula, Carolina Gold, C. I. 2971, Fortuna, Rexoro, and Blue Rose. Other varieties are attacked at times but not so severely as those listed above. The short-grain varieties, as a group, exhibit considerable resistance to the disease. On plants of Vintula and Carolina Gold it is not unusual, at heading time, for the fungus to have attacked the sheaths and thus killed all but the 2 uppermost leaves. Healthy plants of these varieties normally have from 6 to 8 vigorous functional leaves at this period. Invasion of the culm by this fungus seems rather rare. The greatest damage to the plant is due to the reduction of leaf area at a critical stage in the development of the plant.

#### CULTURAL CHARACTERISTICS OF THE FUNGUS

Tissue platings of the lesions in the sheaths have been made each growing season since 1929, and in nearly all cases the same fungus was isolated. From the diseased tissue there is produced a white, rapidly growing mycelium. On corn-meal agar, the colonies, either from tissue platings or from transfers, usually cover the surface of the agar in 2 or 3 days. As soon as spore production begins, the aerial portion of the colony changes rapidly

to a color comparable to Ridgway's French green.<sup>3</sup> On bean-agar or beef-extract peptone agar the colonies remain white. However, sporulation does not occur on these media.

#### IDENTIFICATION AND DESCRIPTION OF THE FUNGUS

The fungus was identified as belonging in the genus *Trichoderma*<sup>4</sup> and has been tentatively designated as *Trichoderma lignorum* (Tode.) Harz.

Mycelium hyaline, septate, 6 to 10  $\mu$ ; conidiophores mostly 6 to 8  $\mu$  in diameter, dichotomously or trichotomously branched, diminishing in size toward the tip; conidia in heads, spherical to subspherical, borne on short verticillate branches that are much constricted at the apex and 2 to 6  $\mu$  in diameter (Fig. 2).

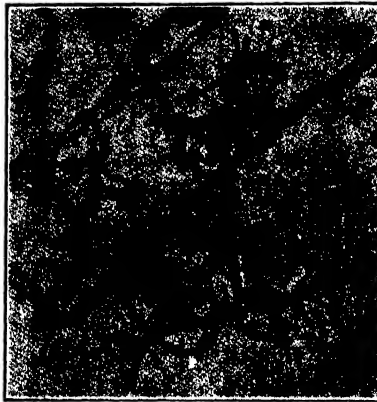


FIG. 2. Mycelium, conidiophores, and spores of *Trichoderma lignorum* from cornmeal agar culture.  $\times 80$ .

#### PATHOGENICITY OF THE FUNGUS

Inoculation experiments were conducted in the greenhouse on plants of the Supreme Blue Rose and Carolina Gold varieties.

The plants were grown in Clarksville silt-loam soil, from the experiment station farm near Fayetteville, Ark., in 3-gallon stoneware jars. About 10 plants of each variety were grown in each of 4 jars. The plants of both varieties in each of 3 of the jars were inoculated, when about half-grown, with conidia from pure cultures of the fungus. Approximately 20 per cent of the plants became infected. The lesions on the sheaths were typical of the disease as it occurs in the field and the fungus was reisolated from the

<sup>3</sup> Ridgway, Robert. (See footnote 2.)

<sup>4</sup> Determination of the genus was made by Miss Vera K. Charles, Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.



infections produced on these plants. No secondary infections were produced on these plants in the greenhouse. The noninoculated plants remained healthy.

#### PATHOLOGICAL HISTOLOGY

Portions of typical lesions on sheaths of Blue Rose rice were placed in strong chromo-acetic fixative for 12 hours, washed, treated with strong hydrofluoric acid for 6 hours, and washed again to remove all traces of the acid. The specimens were then dehydrated in ethyl alcohol, according to the usual procedure, until 70 per cent of alcohol was reached, and further dehydration was accomplished by means of a modified butyl alcohol-ethyl alcohol series, as described by Zirkle.<sup>5</sup> They were then infiltrated with paraffin and embedded. Sections were cut 20  $\mu$  thick and stained with Heidenhain's iron-alum haematoxylin.

An abundance of mycelium was found in the parenchyma tissues in the region of the aerenchyma of the sheaths, and large coarse hyphae of the fungus were found traversing these spaces. The tissues of the invaded areas had been killed by the fungus, as evidenced by their discoloration and the collapse of the cells. The tracheal tissues also were attacked, as shown by the ramification of the mycelium in the vascular elements. There was a greater abundance of mycelium in the tissues on the inner portion of the sheaths.

#### SUMMARY

A previously undescribed sheath disease of rice has been found chiefly in Louisiana and Texas and to some extent in Arkansas.

The disease has been found on most of the commercial varieties of rice grown in Louisiana and Texas. Some varieties exhibit greater susceptibility than others.

The causal fungus is tentatively designated as *Trichoderma lignorum* (Tode.) Harz.

A limited number of inoculation experiments in the greenhouse have demonstrated the pathogenicity of the fungus.

The fungus invades the parenchyma and vascular system of the sheaths and kills the attacked portions.

<sup>5</sup> Zirkle, C. The use of N-butyl alcohol in dehydrating woody tissue for paraffin embedding. *Science*, n.s. 71: 103-104. 1930.

## PHYTOPATHOLOGICAL NOTES

*The Susceptibility of the Peach to Artificial Inoculations with Bacterium Syringae and Some Allied Organisms.*—During the course of some comparative studies of the plant pathogenic bacteria listed in table 1, aqueous suspensions of the organisms were injected into the leaves and succulent shoots of the peach, *Prunus persica*, at various times in 1932, 1933, and 1934.

TABLE 1.—*Organisms used in inoculation experiments to determine the susceptibility of the peach to Bacterium spp.*

Organism	Host	Source	Isolated by
<i>Bacterium syringae</i> .	Lilac	Holland	M. K. Bryan
<i>Bacterium syringae</i> .	Lilac	U. S. A. (Illinois)	M. K. Bryan
<i>Bacterium prunicola</i> ...	Cultivated plum	England	H. Wormald
<i>Bacterium mors-prunorum</i> .....	Cultivated plum	England	H. Wormald
<i>Bacterium papulans</i> ..	Apple (Stayman variety)	U. S. A. (Arkansas)	J. C. Dunegan
<i>Bacterium</i> sp.—Tar-get-canker organism	Apple (Delicious variety)	U. S. A. (Virginia)	J. W. Roberts
<i>Bacterium</i> sp.—Italian prune leaf-spot or-ganism .....	Italian prune	U. S. A. (Arkansas)	J. C. Dunegan

In making the inoculations on the leaves, a hypodermic needle was drawn slowly along the under surface on both sides of the midrib, making two narrow, torn lines in the lower epidermis through which the bacteria were forced into the mesophyll tissue. The symptoms developing from such inoculations appeared within 3 to 5 days and consisted of a very light green (almost white) bleached streak surrounded by purple margins along the needle path. The leaf tissue in the chlorotic streak eventually broke away from the healthy tissue.

The organisms were injected into the twig tissues by carefully inserting the hypodermic needle under the epidermis and forcing the suspension into the tissues. A circular bleached area with a purple margin generally appeared in the immediate vicinity of the needle puncture within 3 days. When the punctures were close together coalescence of the margins gave the twig a very striking appearance.

Adequate controls were maintained for all the experiments and no such symptoms as those just described developed following the injection of the

leaves and twigs with sterile water. As a further control, parallel inoculations were performed with cultures of *Bacterium pruni* and, while symptoms typical for this organism appeared on the leaves and twigs, no pronounced chlorotic symptoms developed.

The results of the inoculation experiments demonstrate that the peach is susceptible to various bacteria listed in table 1 when the bacteria are actually introduced into the tissues of the leaves and twigs. The symptoms indicate a marked effect on the chloroplasts in the immediate vicinity of the point of inoculation and a stimulation of anthocyanin pigment formation in the surrounding regions. That these symptoms were induced by these particular bacteria is demonstrated by their nonappearance in the control experiments.—JOHN C. DUNEGAN, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Department of Plant Pathology, University of Arkansas.

*Alternaria brassicae* as a Parasite of Chinese Cabbage.—For the past decade, Chinese cabbage plants (*Brassica Pe-tsai* Bailey) growing in Massachusetts have been parasitized by an organism similar to an *Alternaria*. However, some uncertainty existed concerning the parasite, so an investigation was undertaken to determine the Latin binomial, the hosts, and the life history of the fungus. This work was terminated and its publication delayed; lately, however, a request has been made for reporting the results.

The manuscript is accompanied by one plate and two text figures and seems unnecessarily long, so it will be retained for reference, in case future questions arise, and only the summary presented here.

Chinese cabbage, in Massachusetts, was seriously parasitized by an organism producing symptoms associated with an *Alternaria* or a *Macrosporium*. A description in literature of *Alternaria* or *Macrosporium* parasitizing Chinese cabbage had not come to the writer's attention. Cultures showed the parasite to be an *Alternaria*. The common name suggested for the disease, therefore, is *Alternaria* leaf spot of cabbage. The species of the organism could not be definitely determined by spore measurements and other morphological characters, since these varied so widely that no standard measurements were at hand. A need for more accurate measurements of conidia with regard to pedicels and beaks is discussed. The species was principally determined by the results of the inoculations. Inoculations within the species and reciprocal inoculations showed that this organism is physiologically identical with *Alternaria brassicae*, which commonly causes the *Alternaria* leaf spot of common cabbage.

This *Alternaria* varied morphologically from descriptions of *A. bras-*

*sicae* as follows: a. The conidia were generally elliptical or fusiform. b. Longitudinal septations were plentiful in the conidia. c. The conidia were blunt or rounded. d. Measurements of conidia showed wider limits of variation:  $5-29 \times 7-102 \mu$ , as compared with  $10-30 \times 35-120 \mu$  for those of *A. brassicae*. e. Some of the conidiophores were wavy. These differences were not considered sufficiently outstanding to establish a separate species or a form as *A. brassicae* f. *Pe-tsai*.

Following is a description of the fungus: *Hyphae*: Long, rather straight, profusely branched when actively growing; sparsely branched in old cultures; cells,  $4-6 \mu$  wide by  $5-30 \mu$  long; at first hyaline to yellow, turning olivaceous to russet and cinnamon. *Conidiophores*: medium length, sometimes tortuous, russet or cinnamon brown; branched or unbranched; each branch arising near the host tissue or substratum, of nearly uniform length, averaging 5 cells,  $4 \times 55 \mu$ ; when supporting single large conidia,  $3.5-7 \times 80-145 \mu$ . *Conidia*: cinnamon brown, no echinulations or rugae; apical portion tapering, rounded to blunt; both catenulate and single; shape, obclavate when catenulate; muriate; longitudinal septations common in those with 5-6 cross walls; limits of variation from dried Chinese cabbage leaves,  $5-29 \times 7-102 \mu$ ; sizes of conidia containing different number of cross walls are reported in table 1, lot 3, and need not be repeated here. *Hosts*: Chinese cabbage and common cabbage, cauliflower. *Noninfectants*: Horseradish, turnip, collards, mustard, brussel sprouts were not infected when inoculated. The parasite is *Alternaria brassicae* (Berk.) Sacc.—W. H. DAVIS, Department of Botany, Massachusetts State College.

*Peach Mosaic*.—Specimens of peach branches were received from the Grand Junction area of Colorado in July, 1932, from Mr. E. W. Bodine of the Colorado Agricultural College, for comparison with the red-suture disease, found in Michigan. These specimens showed little, if any, resemblance to branches of trees affected with red suture. Buds from the Colorado material were grafted into a young Hale peach tree, No. 37-2, in the botany plots at East Lansing. The bud wood was in poor condition and a union appeared doubtful. At the close of the season the grafted buds were dead.

In the spring of 1933, tree No. 37-2 showed a crinkling of the leaves and a mosaic type of mottling on all of the early formed leaves. These symptoms approximated those described by Hutchins<sup>1</sup> for peach mosaic. As the season of 1933 advanced, the symptoms became rather obscure with the new growth appearing normal. However, those leaves that developed during periods of low temperatures, such as occurred at East Lansing between June 12 to 18, 1933, showed marked crinkling and mottling.

<sup>1</sup> Hutchins, Lee M. Peach Mosaic. Abst. Phytopathology 23 (1): 17. 1933.

A number of seedling trees were grafted in August, 1933, with buds taken from tree No. 37-2. Three of these trees were transferred to tubs and removed to the greenhouse in December, 1933. The greenhouse temperatures were high, usually above 75° F. No peach-mosaic symptoms were apparent at these temperatures. Symptoms were finally produced on two trees, one of which was placed close to an outside door in a cooler location and the other in a cool house with approximate temperatures ranging between 40° and 50° F.

In May, 1934, the three trees in tubs were removed outside the greenhouse. Again, those leaves formed during the cool periods showed mosaic and crinkling symptoms, whereas the growth produced in warmer weather appeared normal. All of the other bud-inoculated trees remaining in the field developed characteristic symptoms in 1934. It is thus apparent that peach mosaic has a low temperature optimum for symptom expression. Perhaps this explains Hutchins' statement<sup>2</sup> that the mosaic character of the leaves may be fugitive.—DONALD CATION, Michigan State College, Agricultural Experiment Station, East Lansing, Michigan. Contribution No. 69, Michigan Agricultural Experiment Station.

*Dissemination of Angular Leaf Spot of Tobacco<sup>1</sup> by the Southern Tobacco Worm.*—Rain, accompanied by wind, is probably the usual means of spreading angular leaf spot of tobacco (*Bacterium angulatum* Fromme and Murray) from leaf to leaf and from plant to plant in the field. It has been suggested that insects may be possible carriers of the disease, but positive evidence is wanting. In 1934 several leaves of White Burley tobacco were found with angular-leaf-spot infections in two parallel lines (Fig. 1, A) rather than scattered at random, as is usual (Fig. 1, B). Leaves were then found that were partially eaten by larvae of the Southern tobacco moth, *Phlegethontius sexta*, on which it was possible to trace the angular-leaf-spot infections from the borders of the eaten areas for some distance across the uneaten portions of the leaves (Fig. 1, C and D).

The correspondence in distance between the almost straight parallel lines of angular leaf spot and the width between the abdominal legs of the tobacco worm, and the fact that the abdominal legs are used for clinging to and moving over the surface of the leaf indicate that in this case the bacteria were spread mainly from the legs of the worm. The abdominal legs, 5 sets of 2 each, are equipped with semicircles of hooks that cause slight, though not visible, injury to the leaf. The wounds make excellent infection courts for bacteria, especially if moisture be present. The thoracic legs are

<sup>2</sup> See footnote 1.

<sup>1</sup> The investigation here reported relates to a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

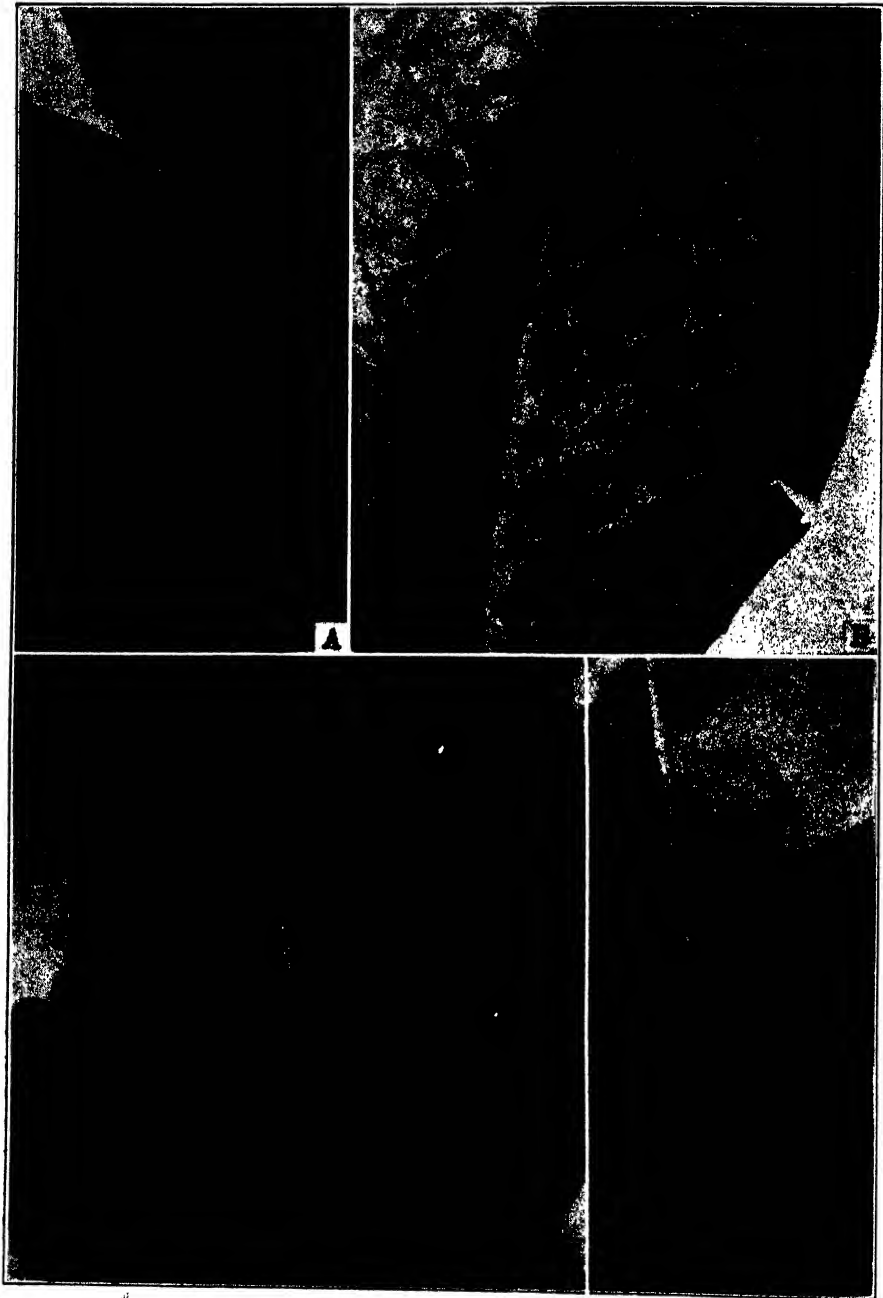


FIG. 1. Angular leaf spot of White Burley tobacco. A. Parallel lines of angular leaf spot on an uneaten leaf. B. Usual random distribution of angular leaf spot. C. and D. Parallel lines of angular leaf spot on leaves of tobacco partially eaten by the tobacco worm.

moved as feelers to the right and left of a straight line and do not seem to be concerned in spreading angular-leaf-spot bacteria.

This method of dissemination of angular leaf spot probably does not occur except when the leaves are damp with dew or rain, so that bacteria can move out of infected areas.—E. M. JOHNSON, Kentucky Agricultural Experiment Station.

*Copper Analysis of Foliage Sprayed with Cuprous Oxide.*<sup>1</sup>—The ideal for a protective fungicide specifies insolubility, because the chemical must remain on the plant for protracted time periods. The insolubility of cuprous (red copper) oxide suggested its use as a foliage protectant.<sup>2</sup> While investigating that problem, this factor of insolubility was rather forcibly demonstrated when an attempt was made to determine quantitatively the copper on sprayed foliage. The cuprous oxide would not come off in the 0.2 per cent nitric acid solution commonly used for removing copper from leaves, although this solution works beautifully with Bordeaux mixture. Obviously, the cuprous oxide is more insoluble in dilute acid than is Bordeaux mixture. To remove the cuprous oxide, overnight immersion is necessary and this frequently results in the extraction of coloring matter from the leaves, which seriously interferes with the subsequent colorimetric analysis. It also requires too much time and glassware.

The addition of 1 per cent of hydrogen peroxide (30 per cent solution) to the 0.2 per cent nitric acid solves the difficulty. Probably the cuprous oxide is oxidized to the cupric form, which is more easily soluble, and a 30-minute immersion is generally sufficient to remove all the cuprous oxide. The usual procedure for the colorimetric analysis of copper in dilute solution can then be followed.—L. L. ISENHOUR and JAMES G. HORSFALL, New York State Agricultural Experiment Station, Geneva, N. Y.

*Physiological Spotting of Pea Seed.*—A spotting of pea (*Pisum sativum* L.) seeds, apparently nonparasitic in origin, has been observed since 1930 on peas grown in Wisconsin, Idaho, Montana, California, Sinaloa, Mexico, and under greenhouse conditions at Rosslyn, Virginia. This spotting has been observed only in the Surprise variety and in closely related types such as Peerless (Improved Surprise), Wisconsin Early Sweet, Early Canner, and in crosses between Surprise types and other varieties.

The spots occur only on the seeds, the pods being normal in every respect. When the seeds have about reached the canning stage, the spots ap-

<sup>1</sup> Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 42, June 14, 1934.

<sup>2</sup> Horsfall, James G. Red oxide of copper as a dust fungicide for combating damping-off by seed treatment. New York (Geneva) Agr. Exp. Sta. Bul. 615. 1932.

pear as small somewhat circular water-soaked areas that later enlarge slightly in size. When the seeds become dry the discolored areas are very conspicuous (Fig. 1), in that they are darker green and in some cases al-

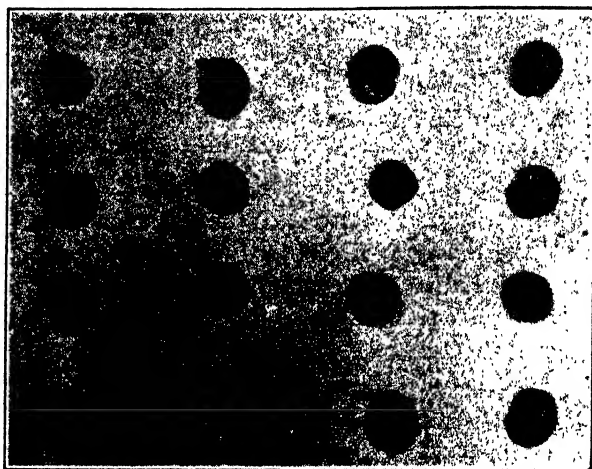


FIG. 1. Lesions showing physiological spotting on seeds of the Wisconsin Early Sweet variety of peas.

most black as compared with the normal green. In general the spotting resembles the lesions caused by the bacterial-blight organism of peas, *Bacterium pisi* Sack., but attempted isolations from such seed have yielded negative results. Spotted seeds are of normal size and germinate just as well as healthy ones. Not all the pods on a plant produce spotted seeds; neither are all the seeds in a pod necessarily affected. In fact, in some cases only one seed in a pod may show the spotting, while in other instances all the seeds may be affected.

Normal seed may, under favorable environmental conditions, produce spotted progeny. On the other hand spotted seeds do not always produce spotting in their progeny but may do so if conditions are favorable. Seedsmen have reported heavy hand-picking costs in areas where this disorder commonly occurs. Hand picking is not recommended as a method of control, but only for improving the appearance of the seed stock.—W. J. ZAUMEYER and B. L. WADE, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

*Internal Breakdown of Pea Seed.*—In the spring of 1934 dry seed of Laxtonian peas, *Pisum sativum* L., was received from Salinas, California. Of this seed about 20 per cent showed discolored areas surrounding the cen-



ters. The lesions are spherical in shape, irregular in outline, from 1 mm. to 5 mm. in diameter, and vary in color from a dull light brown to dark brown (Fig. 1). Attempted isolations of a suspected causal organism gave

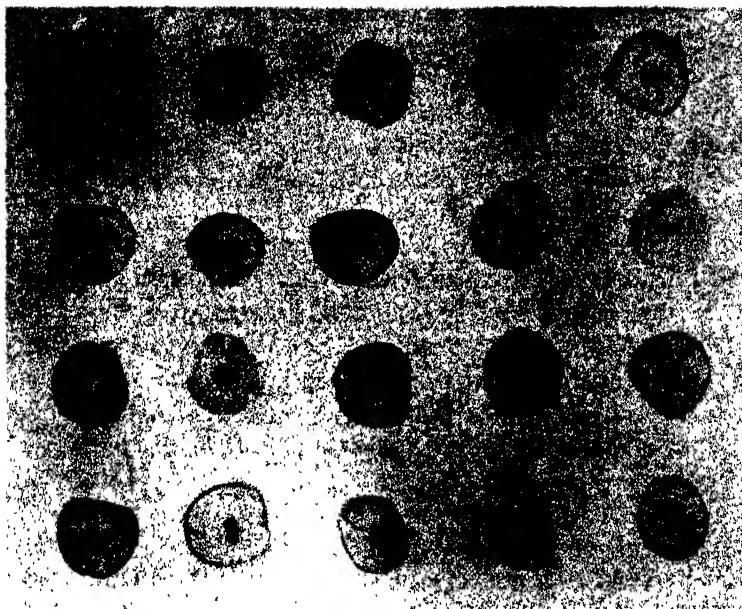


Fig. 1. Cross-sections of pea seeds showing internal breakdown.

negative results. From a germination test it was evident that the internally discolored seeds germinated as well as normal seeds from the same stock. The seed was harvested near Salinas in June, 1933, during relatively cool weather. The available records indicate that an excessive amount of water was supplied to the field during the ripening period.

This condition may be the same as that described by de Bruijn,<sup>1</sup> who explains it as the interaction between slow ripening and the occurrence of marsh areas in the field. She found among other things a decrease in sugar and soluble nitrogen and an increase in starch and insoluble nitrogen. De Bruijn's article covers the situation adequately but, inasmuch as it is not generally available to American readers, it was thought advisable to publish this brief note, so that seedsmen, pathologists, and other interested individuals would know of its occurrence in the Salinas region of California and be on the watch for it in other sections of the United States.

<sup>1</sup> Bruijn, H. L. G. de. Kwade harten van de erwten. *Tidjschr. Plantenz.* 39: 281-318. 1933. Krankhafte Erscheinungen ab Erbsensaat. *Abst. in Die Ernährung der Pflanze* 30: 68. 1934. [Abst. in *Rev. Appl. Mycol.* 13: 204-205. 1934.]

Seedsmen and growers have been somewhat apprehensive that the internal breakdown might be associated with some seed-borne disease. Since the affected seeds appear to be entirely normal externally, it is impossible to remove such seeds by hand picking.—B. L. WADE and W. J. ZAUMEYER, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

*Leaf Smut of Rice in the United States.*—Leaf smut of rice caused by *Entyloma oryzae* H. and P. Sydow, first described on rice from the Philippine Islands, is now known to occur in Japan and in the lower Mississippi Valley rice-growing areas in the United States.

Recently, S. Ito and his coworkers have cultured a fungus identical with *Entyloma oryzae* from leaf spots formerly attributed to a fungus known as *Sclerotium phyllachoroides* Hara and *Ectostroma oryzae* Sawada.

Specimens of this leaf spot received by the writer from E. Kawamura of Japan are identical with the small black leaf spot on rice in the United States. Examinations of these leaf spots on rice from Arkansas, Louisiana, and Texas have shown that there are present in the older lesions chlamydo-spores that, in all essential details, are identical with those given in the original description of *Entyloma oryzae* in *Annales Mycologici* 12: 197. April, 1914.—E. C. TULLIS. (Cooperative investigation between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Arkansas, Louisiana, and Texas agricultural experiment stations.)

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